BCBC2020-01 Protected coral reproduction

Literature review, recommended study species, and description of spawning event for *Goniocorella dumosa*

Prepared for Department of Conservation

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Cover image: Schematic of a spawning branching stony coral colony [Erika Mackay NIWA].

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Executive summary

Reproductive strategy (alongside age, growth, mortality), determines the productivity of a coral species or population, and combined with information on dispersal, reflects this groups capacity to recover from trawling impacts. Reproductive strategies employed by deep-sea corals however, are generally poorly documented. An improved understanding of all aspects of coral reproduction will improve our knowledge of population connectivity, the vulnerability of species to fishing impacts, and their ability to recover from these disturbances. This is critical for supporting a long-term plan to manage and conserve populations of protected corals for the New Zealand region.

This study summarises recent relevant knowledge of reproduction for protected deep-sea and cold-water corals in New Zealand and for related species for which reproductive studies have been carried out globally. While there have been some recent advances in knowledge in this field, and the research described in our literature review has highlighted new observations of spawning behaviours, reproductive modes, and fecundity estimates for key deep-sea coral groups, there remains large knowledge gaps for many species globally.

In this project, the review showed that there is variability in spawning frequency, from periodic to quasi- continuous, and species that show continuous spawning or gametogenesis may still be influenced by environmental cues. A range of reproductive modes occur among even closely related corals, highlighting the need for species specific studies. For example:

- Within the scleractinian (stony) branching corals, both broadcast spawning (*Solenosmilia variabilis*, *Madrepora oculata*) and brooding (*Goniocorella dumosa*) occurs.
- Antipatharians (black corals) appear to be a strictly gonochoric (male or female) group with the exception of one species, *Stichopathes saccula*.
- Some primnoid gorgonians (octocorals) such as *Fannyella* and *Thouarella* brood their young.

Information compiled from an interrogation of the NIWA Invertebrate Collection (NIC) database *niwainvert* has enabled the production of a prioritised list of suitable samples for a New Zealand reproductive study, based primarily on histology. Study species recommended are the scleractinian stony corals *Desmophyllum dianthus*, *Goniocorella dumosa*, and *Enallopsammia rostrata*, and the gorgonian octocorals *Paragorgia arborea* and *Primnoa notialis.* Several of these species were ranked under 'productivity' as medium and/or high-risk groups in a recent semi-quantitative pilot ecological risk assessment. The number of samples currently available in the NIWA Collection in appropriate fixative (particularly those recently collected at-sea), as well as those corals that could be used for regional comparisons, also aided the species prioritisation process. We recommend considering the New Zealand endemic alcyonacean soft coral *Taiaroa tauhou* for a reproductive study as a small unique collection of samples is available.

Liaison with international experts is ongoing to progress a collaborative study for several nominated species listed above. Future research is needed to describe and quantify the baseline reproductive biology and output at both the colony and population level for the selected New Zealand protected corals.

The preliminary results of a recent opportunistically observed spawning event by *G. dumosa* in NIWA's Marine Environmental Manipulation Facility (MEMF), are summarised. This was the first

observation of live spawning by a deep-sea coral in New Zealand, and as such represents a major milestone in the field of deep-sea coral reproduction, changing our understanding of the reproductive mode and seasonality of spawning for this species. Previously the species was described as gonochoric (i.e., separate sexes) broadcast spawners. We now know that for this species larvae mature within the coral polyp's calyx or cup region, and thus is a brooder. Further research is recommended to determine the spawning triggers and settlement cues for this species.

Detailed reproductive descriptions based on the examination of a sample of histology slides for adult *G. dumosa* sampled in September 2020 are presented. Histological section images confirmed the in-aquaria and microscopic observations, that this species is a brooder. It was indicated that individual colonies of this coral are single sexed, and the sex ratio would be approximately one to one. Both males and females exhibited gametogenic material at a range of development stages within individual polyps, from reasonably immature to fully mature. This means that *G. dumosa* is most likely to exhibit a reproductive strategy somewhere between a serial spawner and a quasi-continuous spawner in which individuals brood over a protracted 'season' of the year. This reproduction strategy is further supported by the observations that males showed reasonable consistency between individuals, exhibiting maturing spermatocytes and fully developed spermatozoa. This histological evidence ties in with experimental observations of spawning from September to November 2020. The total fecundity per polyp could not be ascertained from this limited data set of histology preparations. We observed advanced larvae still retained moderate reserves of lipid globules which would sustain the larvae, post-release, until the larvae can settle and develop fully functioning feeding apparatus. Our findings are compared with an earlier study that described *G. dumosa* reproduction, resulting in a more informed understanding of the reproductive behaviour utilised by this coral.

Finally, recommendations are made for further research that may improve our understanding of reproductive strategies for several coral groups, with the aim of providing improved information on population characteristics and susceptibility to the effects of fishing for protected species populations, as outlined in the CSP Protected Coral Medium Term Research Plan.

1 Introduction

In order to support a long-term plan to manage and conserve populations of protected corals in the New Zealand region, an understanding of reproductive strategies for a range of key coral taxa is required. Detailed knowledge of productivity in deep-sea corals (age, growth, reproduction), is generally lacking. Currently there are limited data describing the known spawning period, fecundity, and individual and population level variation in reproductive output for the various New Zealand protected coral groups. There is also a poor understanding of larval settlement cues and settlement time.

It is important to improve our understanding of how population connectivity, in part driven by coral productivity and fecundity, determines the vulnerability of species to fishing impacts and their ability to recover from these disturbances. As such, data is required in the following areas:

- Improved understanding of reproductive and dispersal capacity, i.e., linking life history traits such as reproductive mode and seasonality with dispersal potential;
- Increased knowledge of larval motility, behaviour and duration to inform potential dispersal distance.

This report relates to corals that are protected under the New Zealand Wildlife Act 2010 (amendment of Schedule 7A of the Wildlife Act 1953) which are the Orders Antipatharia (black corals), Alcyonacea (those gorgonian octocoral groups previously known as Gorgonacea, Scleractinia (stony corals), and the hydrocoral(lace coral) family Stylasteridae.

A semi-quantitative pilot ecological risk assessment for protected corals (Clark et al. 2014) considered various sources of information and data available for the age, growth, reproduction, colonisation, and dispersal of protected corals. This information was used to rank the "productivity" of a coral species or group, which also considers its ability to recover from trawling impacts. However, as noted by the authors, such information was scarce as unlike their shallower counterparts, the reproductive strategies employed by deep-sea corals are generally poorly documented. These reproductive strategy data are also useful to inform population scale reproductive models and to establish an understanding of regional scale gametogenic variability (Fountain et al. 2019).

A lack of such information for corals was also highlighted in the report from the Conservation Services Programme (CSP) Protected Coral Workshop held in October 2017 (Hjorsvarsdottir & Tracey 2017), and as well as being noted as a significant gap in the 'State of Knowledge of Corals Report' (Tracey & Hjorsvarsdottir 2019). While more information is available for shallow-water corals, the reviews indicated that reproductive information is certainly lacking for the deeper dwelling corals, locally as well as globally. The reviews also supported the findings of the aforementioned risk assessment (Clark et al. 2014).

The literature on New Zealand deep-sea coral reproduction studies included in and published after the review by Consalvey et al. (2010) and the State of Knowledge Report (Tracey & Hjorvarsdottir 2019), is reviewed. We build on the content of the two earlier reviews and while the focus was on species found in the New Zealand region, we include international coral reproduction studies relevant to our protected coral groups. We also examined the availability, via a database exploration, of preserved coral specimens suitable for a reproductive study. From this we provide recommendations as to which species could be a focus of further reproduction studies. To help meet the deliverables of this project, liaison with an International coral expert in this field has been on-

going - co-author Rhian Waller from the University of Maine, United States. Continued collaboration with this team will add value to the recommended protected coral study. The recent observations of a spawning event by *Goniocorella dumosa* colonies in NIWA's Marine Environmental Manipulation Facility (MEMF), are also briefly described, and proposed next steps for this work are summarised. The reproductive development described from examined histology slides inform adult reproductive mode for this coral, a species that was previously considered a broadcast spawner, now known as a brooder.

There are strong synergies in this research with previous and current research projects that examine coral age and growth -Marriott et al. 2020 (POP2017-07); protected coral connectivity, Bilewitch & Tracey 2020 (POP2018-06); coral biodiversity in deepwater fisheries bycatch, Macpherson et al. 2020 (INT2019-05), and particularly previous work addressing larval dispersal and connectivity (Holland et al. 2020; Zeng et al. 2017; 2020). Additionally, this research feeds into coral recovery studies (e.g., Fisheries New Zealand Project ZBD2020-07 Recovery of Seamount Communities), and any future benthic risk assessments.

The research addresses Objective E of the current DRAFT CSP Protected Coral Plan that states: 'Adequate information on population level and susceptibility to fisheries effects exists for protected species populations identified as at medium or higher risk from fisheries.'

2 Objectives

The overall objective of this work is to determine the reproductive strategies of key protected deep-sea corals in the New Zealand region, and to document coral spawning of *Goniocorella dumosa* colonies previously held in aquaria at NIWA in the MEMF. An understanding of reproductive strategies is an essential requirement to assist with the management and conservation of these protected species. Reproduction is one source of information (alongside age and growth, colonisation, and dispersal) that is used to determine the productivity of a coral species or group, which in turn reflects its ability/inability to recover from anthropogenic impacts such as trawling. This study, therefore, will review both literature and empirical data available for reproduction of New Zealand corals.

The Specific Objectives of Project BCBC2020-01, Protected coral reproduction, are:

1. Summarise the literature on deep-sea coral reproduction studies included in and published after the review by Consalvey et al. (2010) and the State of Knowledge Report (Tracey & Hjorvarsdottir 2019).
2. Examine preserved coral specimens currently held in the NIWA Invertebrate Collection to assess what amount of reproductive information can be obtained from stored samples.
3. Select which of the key protected coral groups will be a focus of further reproduction studies.
4. Monitor progression of, record and provide a report of the 2020 *G. dumosa* spawning event in NIWA aquaria, including a written account and imagery of observed life history traits of larvae and polyps (e.g., fecundity, larval swimming and feeding behaviour, pelagic larval duration (PLD) settlement behaviours and cues, sequential developmental biology imaging) and a life-cycle graphic for DOC educational resources.

An additional aim of this project was to liaise with international coral reproduction experts, in order to share expertise and collaborate, and be able to contextualise the results for New Zealand more broadly, e.g., carry out regional comparisons.

3 Methods

3.1 Review the literature on coral reproduction strategies

Factors that affect the dispersal and recruitment of coral larvae, including life history strategies, larval longevity, relevant settlement cues and substrate suitability, are all important information to understand coral productivity to feed into any ecological risk assessment (Tracey and Hjorvarsdottir, 2019). There are, however, few data available for the New Zealand regions protected coral groups. Previous reviews by Consalvey et al. (2006) and Tracey & Hjorvarsdottir (2019) summarised deep-sea coral reproduction studies primarily from the New Zealand region, but included some relevant global studies for taxa in common.

We briefly describe the key findings from these two reviews for protected coral groups in New Zealand waters, building on the content of the two earlier reviews and specifically where Chapter 2 in Tracey et al. (2019), presented a summary for New Zealand and International coral reproduction studies relevant to protected coral groups. We add relevant and more recent literature not originally included by the report authors (see below and Table 4-1). For some groups, a range of , reproductive modes, larval behaviour, and fecundity estimates exist, but there are large knowledge gaps for many species groups globally.

Descriptions of the terms used in the report are provided in a Glossary.

3.2 Examine preserved coral specimen records currently held in the NIWA Invertebrate Collection

An examination of records of suitably-preserved coral specimens held in the NIWA Invertebrate Collection (NIC), was carried out. The NIC Specify database *niwainvert* was interrogated to assess what reproductive information can be obtained from stored samples and the focus was on two protected coral groups, stony corals and gorgonian octocorals. The NIC also holds some samples of black coral and stylasterid hydrocorals in formalin but we limited our examination to two groups to focus in on what could be feasibly be achieved in a reproductive study.

Priority was to assess the samples available that had been stored in formalin, the standard fixative for histological work. The optimal sample storage method for histological methods is to first fix the sample in formalin, then transfer it to ethanol for long-term preservation. Ethanol fixed organisms can also be used for histology, but this preservative method is not ideal. Samples included in the extract were the stony branching coral species targeted for potential up-coming histology work and collected appropriately during very recent voyages to the Chatham Rise.

3.3 Select key protected coral species for a reproduction study

From the examination of preserved sample records, a data mining exercise was carried out that considered both the species and their corresponding metadata to help with recommending the groups to consider for a reproduction study using histological methods. Key information considered to aid the prioritisation of the recommended study species included:

- numbers available by species – a single polyp and / or numerous polyps per colony,
- length of time the samples been stored in preservative (formalin),

- fixation, prioritising those samples that had been fixed in formalin then transferred reasonably quickly post collection into 80% ethanol (the ideal scenario),
- what species had been listed in a semi-quantitative pilot ecological risk assessment in 2014 as a medium and/or high-risk group,
- what species had been successfully studied by the coral expert Rhian Waller that could increase our confidence in obtaining reproductive data for a particular species and also enable a regional comparison.

A summary table was then prepared listing species, count of samples, count of the number of individuals available, and a count of those that had been collected post 2010 for a recommended study. The recommended taxa for a reproduction study were highlighted.

3.4 Monitor progression of, record and provide details of the 2020 *Goniocorella dumosa* spawning event

The release, development, and settlement of the larvae released by adult colonies held in aquaria were monitored visually. Information collected post the spawning event included swimming behaviour, changes in morphology, settlement times, and settlement medium. Histology slides were examined to describe the reproductive stages.

3.4.1 Spawning event of the species *Goniocorella dumosa*

Sample collection: In June 2020, colonies of *Goniocorella dumosa* were collected by beam trawl from ~400 m water depth on the Chatham Rise, as part of a research programme on the "*Resilience of Deep-sea Benthic Communities to the Effects of Sedimentation"* (ROBES) (Clark et al. 2021). Colonies were held in an on-board flow-through aquarium system onboard the R.V. *Tangaroa* for two days before being transferred into holding tanks in the MEMF.

Observations: In the MEMF, the coral colonies were kept in the dark, in flow-through tanks (seawater from the adjacent bay filtered to 0.1µm) and chilled to 8 ^{o}C to replicate the temperatures recorded *in-situ* on the Chatham Rise. On 17th September 2020, during respirometry trials at the end of a four-week sediment tolerance experiment on the coral colonies, larvae (also referred to as planulae) of *G. dumosa* were observed mid-water inside a respiration chamber (Figure 3-1).

Figure 3-1: *Goniocorella dumosa* coral colony in the respiration chamber in the MEMF in which some polyps spawned and released fully formed free-swimming larvae.

Larvae were subsequently found in several other sediment tolerance chambers and in a holding tank containing a number of coral colonies that had been held since at-sea collection but had not been used in the experiment.

The larvae were collected by pipette and placed into small holding tanks. Small coral fragments were placed into larval tanks to provide a possible substrate for settlement. They were checked and fed daily with a 3 mL mixture consisting of 10% commercial coral food (JBL Koralfluid, Neuhofen, Germany) and 10% commercial shellfish diet (larval shellfish diet 1800, Reed Mariculture, Campbell, CA, USA), that was diluted to the required volume with filtered seawater (FSW).

Over a three-month period, we documented the behaviour and settlement of larvae and the development and calcification of corallites. Beaumont et al. (in prep.), will provide a more detailed description of the settlement and development of *G. dumosa* corallites.

3.4.2 Histology

Histology samples were prepared as part of a *G. dumosa* sediment tolerance experiment within NIWA's ROBES Programme to assess the impact of sediment on its internal tissue and organs, including reproductive organs (Mobilia, submitted). While the samples have been prepared from organisms held in aquaria, rather than from samples in the NIC and / or freshly preserved at

collection from the Chatham Rise, it was decided that they would be a useful resource to obtain reproductive information for a particular time period, (August to September).

Histological sections were prepared from intact coral polyps. Histological sections were prepared from intact coral polyps. A total of 100 slides from 24 individual colonies were examined to produce descriptions of the developmental reproductive stages for *G. dumosa*. The sections had been prepared to look at the possible detrimental effect of suspended sediment on coral colonies and so were not optimal for the assessment of reproductive state and total fecundity. Few sections, through a polyp will only show a proportion of the developing reproductive tissue of that polyp and ideally serial sectioning is required. This means that the total fecundity of the polyp could not be assessed and that potentially not all developmental stages of reproductive tissue within the polyp were observed. An optimal sampling strategy over time would require serial sections to be made through the entire polyp, and a larger sample of individual coral colonies examined to robustly characterise spawning behaviour.

Histology slide preparation: Coral fragments were fixed in 10% neutral buffered formalin for 48 hours. The fragments were then transferred into 70% ethanol (ETOH) until the decalcification step outlined below took place. Two polyps were sampled from each coral fragment for decalcification and subsequent histological processing. Polyps were decalcified in 5 % hydrochloric acid (HCl) for 2 hours or until the skeleton was dissolved, rinsed in distilled water to remove any residual acid, and then stored in 70 % ETOH until histological preparation.

Samples were then dehydrated through a series of ethanol concentrations (70, 90, 100 x 2 changes), transferred to a clearing agent (Xylene), and embedded in paraffin wax. Polyps were embedded in paraffin blocks, sectioned longitudinally (whole polyps) to 6 µm using a rotatory microtome (Leica Biosystems RM2235), mounted on glass microscope slides, stained with haematoxylin and eosin (H&E), and coverslips placed over the section.

Polyp sections were observed and photographed using a research grade compound light microscope (Nikon Ni-e) and digital images were taken with the integral Nikon digital camera (DS-Ri2) at 10x - 400x magnification.

4 Results

4.1 Literature review

From the literature review it was clear that for some coral groups, a range of spawning behaviours, reproductive modes, and fecundity estimates exist, but globally there are large knowledge gaps for many other deep-sea or cold-water corals. Few studies have been carried out in New Zealand (n= 5 including the study described herein, Table 4-1), and more international studies exist, but there remains a poor understanding of deep-sea coral reproduction. Due to the lack of published information from New Zealand studies, we have reviewed studies globally but only those which included species and genera which are known to occur in New Zealand waters. Reproductive information is summarised below by protected coral group. A Glossary of Terms is provided (Section 4.1.1), and the terms are also highlighted throughout the review.

4.1.1 Glossary of terms

actinopharynx	invagination of the epidermis to form a short muscular tubular passageway between the mouth and gastric cavity in a polyp, mostly lined with flagellated supporting cells
ahermatypic	describes non-reef forming coral species
atresia	absence or disappearance of an anatomical part by degeneration
asexual fission	a form of asexual reproduction whereby the parent polyp or colony is split into two individuals (rather than growing from the parent polyp or colony, as is the case in *asexual budding*)
azooxanthellate	refers to corals that lack photosynthetic symbiotic algae, *zooxanthellae*, in their polyps; azooxanthellate corals must therefore obtain required nutrients by capturing food particles from the surrounding water column with their polyp tentacles
brooding	a reproductive mode whereby gametes are fertilised (or produced asexually) and develop internally (i.e., within the adult coral) into larvae before being released into the surrounding water
budding	a form of asexual reproduction that occurs when a portion of the parent colony pinches or "buds" off to form a new individual; budding can be either intra-tentacular (buds form from the parent polyp's oral discs, producing a new polyp within the parent polyp's ring of tentacles) or extra-tentacular (buds form outside the parent polyp's ring of tentacles, producing a smaller polyp)
congeneric	describes distinct species within the same genus (e.g., *Fannyella rossii* and *Fannyella spinosa*)
conspecific	describes individuals within the same species
ectoderm	the outer germ layer of cells in an embryo that gives rise to the outer covering of a polyp; the outer layer of pluripotential cells in the embryo, after establishment of the primary germ layers during the gastrula stage of development

endoderm	the inner germ layer of diploblastic and triploblastic embryos that gives rise to internal tissues such as the actinopharynx; the inner layer of pluripotent cells in the embryo, after establishment of the primary germ layers during the gastrula stage of development
endodermal cellular mass	mass of cells residing within the ectoderm of a developing larva, this will differentiate into the endoderm and other internal tissue constituents as the larva develops
fecundity	reproductive potential; for corals, fecundity is typically reported as either the number of oocytes or planulae per polyp
flagellum (plural flagella)	single, elongate motile structure consisting of nine pairs of microtubules around two single central proteinaceous microtubules extending from the apical surface of an epithelial cell or tail of spermatozoan
gamete	general term for reproductive cells
gametogenesis	process by which gametes are formed and mature
gonochorism (adj. gonochoric)	describes coral species in which polyps and/or colonies are either male or female
hermaphroditism (adj. hermaphroditic)	describes coral species in which both male and female gametes are produced within a polyp or colony; corals can exhibit either: sequential hermaphrodism, whereby polyps and/or colonies change their sex (i.e., from male-to-female or female-to-male); or, simultaneuous hermaphroditism, whereby male and female gametes are produced simultaneously by a coral polyp or colony
hermatypic	describes coral species which secrete calcium carbonate skeletons to form reefs
larva (plural larvae)	general term for the immature stage in a marine invertebrate's life cycle which has developed from a fertilised oocyte and eventually settles on the seafloor for growth; in corals, this stage is termed a planula
lecithotrophic	refers to the larval nutritional mode whereby larvae feed on yolk prior to settling
lumen	the inner open space or cavity of a tubular organ, such as the gastro-vascular cavity of a coral polyp
mesentery	internal longitudinal partition of tissue providing structural support and increasing surface area, which is important in nutrition and fertility of anthozoans. A mesentery develops by infolding of the mesoglea and its lining gastrodermis from the body wall of the polyp. Multiple mesenteries are arranged radially within the gastrovascular cavity of the polyp (between the septa in scleractinian corals) and are attached to the oral disk. Termed a complete mesentery if joined with the actinopharynx
mesoglea	the connective tissue of coral and all cnidarians consisting of collagenous fibres embedded in a gelatinous material or ground substance of highly hydrated protein and neutral polysaccharide polymers and containing amoebocytes and other cells. The proportion of matrix to fibre and cells in this layer varies with the species and its condition

oocyte	female reproductive cell (also see: gamete) which undergoes vitellogenesis
oogenesis	the development of oocytes from immature germ cells (oogonia)
oogonium (plural oogonia)	the primordial cell from which an oocyte originates, first stage in oogenesis
pelagic larval duration	the length of time between spawning/larval release and settlement
planktotrophic	refers to the larval nutritional mode whereby larvae feed on plankton prior to settling
planula (plural planulae)	free-swimming larva of a cnidarian; can be lecithotrophic or planktotrophic
productivity	as used in this report and a pilot ecological risk assessment for New Zealand protected corals (Clark et al. 2014), productivity refers to the overall potential for a species to recover from impacts caused by human disturbance (e.g., fishing)
reproductive mode	refers to the method by which a species reproduces (i.e., spawning vs. brooding)
reproductive periodicity	describes the frequency of reproductive spawning or brooding; reproductive periodicity can be continuous (gametes and/or planulae are released year-round), quasi-continuous (gametes and/or planulae are released several times annually), or seasonal (gametes and/or planulae are released seasonally); alternatively, a species may exhibit little to no reproductive periodicity, where reproduction is triggered instead by an environmental cue (e.g., an increase in food availability or change in temperature)
reproductive strategy	general term used to collectively describe the overall reproduction methods of an individual or species (i.e., asexual and/or sexual reproduction, brooding or spawning, and reproductive periodicity)
septum (plural septa)	one of the vertical calcareous plates or partitions radiating from the corallite wall toward the central axis within the calyce that provide support to the mesenteries
settlement	the point when an individual first takes up permanent residence on the substratum. In sessile species this is when the planktonic propagule (e.g. larvae) has cemented itself to the surface
spawning, broadcast	a reproductive mode whereby individuals release gametes into the water column to undergo external fertilisation and development
spawning, periodic	a reproductive mode that can occur at different timeframes
spawning, continuous	A reproductive mode that can be continuous, the gametes and/or planulae are released year-round
spawning, quasi-continuous	a reproductive mode when gametes and/or planulae are released several times annually

spawning, seasonal	a reproductive mode when gametes and/or planulae are released seasonally. Note spawning can also be triggered by an environmental cue (e.g., an increase in food availability or change in temperature)
spermatocyte	immature male reproductive cell, second stage in spermatogenesis
spermatocyst	clusters of sperm or spermatocytes clearly enveloped by a membrane like cyst
spermatogenesis	the development of sperm from immature germ cell to spermatazoa
spermatogonium	immature male reproductive cell, first stage in spermatogenesis
spermatozoa	mature motile male sex cell that contains the genetic information to be transmitted by the male, final stage in spermatogenesis (also see: gamete)
stomodeum	mouth and actinopharynx as it begins developing in the coral embryo and planula, is also often applied to the actinopharynx of the adult
tentacle	hollow, contractile extension of the polyp's oral disk distal to the mesenteries, typically cylindrical, commonly tapering to a point but in some species terminating in a spherical acrosphere, and rarely branched. In octocorals, each tentacle has two diametrically arrayed rows of short pinnules. The tentacle's internal cavity is continuous with the gastrovascular space, continuous with that of the main body. In most species, it is studded with nematocysts and/or spirocysts, either scattered or arrayed in batteries. Tentacles are typically used in food capture, for defense, and sediment removal; in some species, some tentacles are specialised to take up dissolved organic matter from seawater
vitellogenesis	formation of the yolk and its accumulation in the yolk sac

4.1.2 Branching stony corals (Order Scleractinia)

Studies from both the North East (NE) Atlantic and South Pacific region, including New Zealand, indicate the hermatypic branching stony corals *Lophelia pertusa* (referred by some as *Desmophyllum pertusum*; see Addamo et al., 2016), *Solenosmilia variabilis*, *Madrepora oculata*, *Goniocorella dumosa*, *Oculina varicosa*, and *Enallopsammia rostrata* are **gonochoric broadcast spawners** (Brooke, 2002; Brooke and Young, 2003, 2005; Burgess and Babcock, 2005; Waller and Tyler, 2005; Brooke & Järnegren 2013, Larsson et al., 2014).

In New Zealand waters the reproductive modes of *S. variabilis*, *M. oculata*, *G. dumosa*, and *E. rostrata* are thought to exhibit seasonality, with fertilisation occurring at the end of summer in April/May in synchrony with increased food availability (Burgess and Babcock, 2005). Similarly off the East coast of Florida, the gametogenic cycle of *O. varicosa* colonies appears to begin in early summer, with spawning occurring during late summer/fall (Brooke and Young, 2003). No comparable studies have been carried out on the reproduction of *O. virgosa*, a congeneric species found in New Zealand and New Caledonian waters, and so it is uncertain whether this species exhibits a similar reproductive cycle to *O. varicosa.* Additional research is required to confirm the prediction that these are broadcast spawners, as it is possible that some species may instead be **brooders** (Burgess and Babcock, 2005; see this report section 6, where *G. dumosa* was shown to be a brooder rather than broadcast spawner as previously thought).

In a **brooding** coral, larvae mature within the coral polyp's calyx or cup region and then crawl or swim away fully developed as **planulae** into the surrounding water. Eventually the larval recruits

settle on suitable substrate which can include branches of the parent colony, in an area not covered with live coenenchyme tissue (basally) (S. Cairns and M. Kitahara, pers. comms.). This is in contrast to spawning corals that release **gametes**, not larvae, into the surrounding water. Gametes are then fertilised externally, and larvae may swim for a period (i.e., hours to weeks) before settling on the substrate for further growth and development. Generally, broadcast spawners are thought to disperse over greater distances than brooders, as brooded **larvae** can be immediately ready for **settlement (as defined in Connell 1985)** , and therefore information on a species' reproductive mode can greatly inform our understanding of their productivity and connectivity. However, it should be noted that the larval behaviours (i.e., swimming vs. non-swimming larvae), nutritional modes, and settlement cues of spawning and brooding deep-sea scleractinian corals are almost completely unknown, both globally and in New Zealand waters, as there have been very few *in situ* observations of deep-sea scleractinian larvae (see Table 4-1).

Some scleractinian species undergo both sexual and asexual reproduction. Evidence of asexual reproduction in the form of **budding** has been observed from colonies of *G. dumosa* and *S. variabilis* on the Chatham Rise (Burgess and Babcock, 2005; S. Cairns, pers comm). A recent genetic analysis from several Australian seamounts indicates that asexual budding may actually be the dominant mode of reproduction for *S. variabilis*, which was proposed as the reason for limited dispersal of this species when compared to the solitary scleractinian cup coral *Desmophyllum dianthus*, which appeared to have a much greater dispersal potential (Miller and Gunasekera, 2017) and which can occur as non-genetically related clusters of individuals (Holland et al., 2020). In a New Zealand study, Zeng et al. (2017) suggested that *S. variabilis* may have a higher inbreeding rate than two other scleractinian species, *M. oculata* and *G. dumosa*, supporting the prediction that *S. variabilis* undergoes both asexual and sexual reproduction.

Most deep-sea stony branching corals are hypothesised to produce **lecithotrophic** larvae which feed on yolk as opposed to **planktotrophic** larvae which feed on plankton in the surrounding waters (e.g., Brooke and Young, 2005; Burgess and Babcock, 2005; Tracey and Hjorvarsdottir, 2019, and references therein); however, larval feeding mode cannot be known for certain without direct observations. Indeed, Larsson et al. (2014) observed that *L. pertusa* colonies in aquaria produced planktotrophic larvae, even though it was previously hypothesised that this species produced lecithotrophic larvae due to potential food limitations (Waller and Tyler, 2005), and more recent studies have confirmed the larvae feeding on small particles (Strömberg & Larsson, 2017). Based on their small size and active swimming behaviour, *O. varicosa* larvae may also be planktotrophic (Brooke and Young, 2003); although Brooke and Young (2005) suggest the successful development of *O. varicosa* in experimental conditions also indicates potential lecithotrophy. Laboratory and/or *in situ* observations of the New Zealand species *S. variabilis*, *M. oculata*, *G. dumosa*, and *E. rostrata* are needed to determine their larval nutritional modes. This information will be useful for determining the vulnerability of these species, as lecithotrophic larvae may have increased survival from predation (Mercier et al., 2013) in food-limited environments compared to planktotrophic larvae.

Figure 4-1 illustrates life-history modes of both broadcast spawning and brooding used by branching scleractinian stony corals.

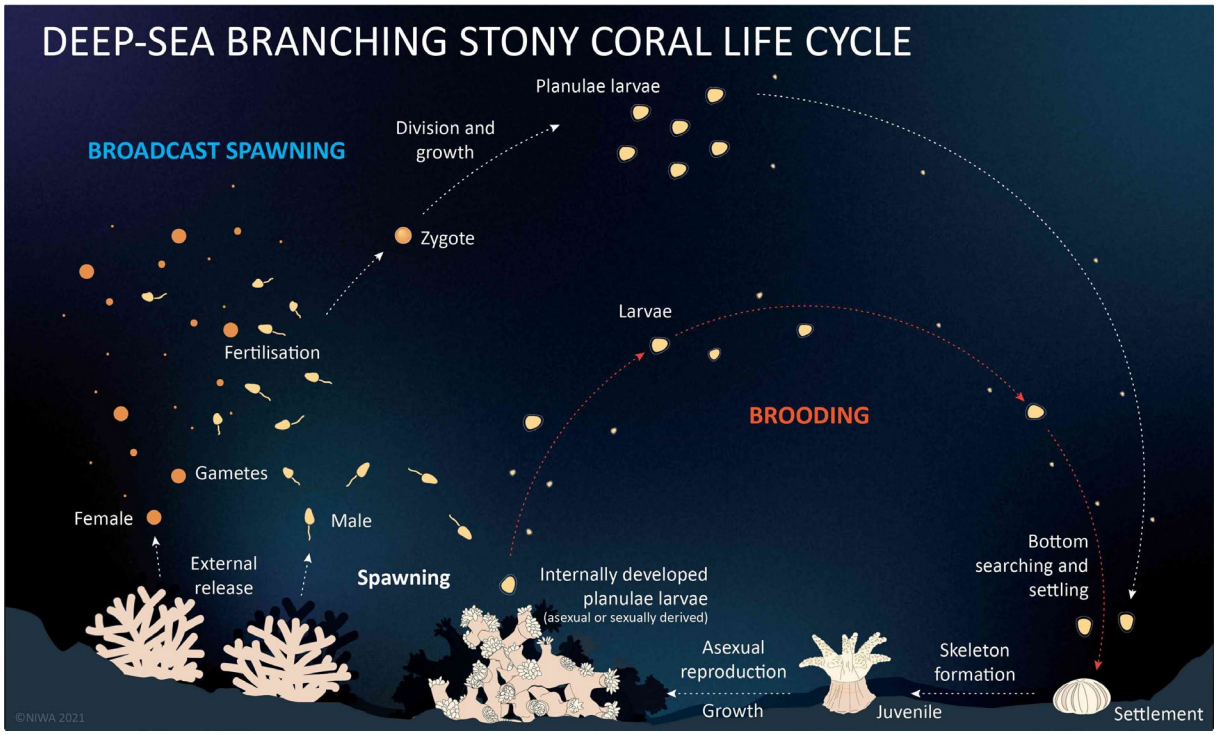

Figure 4-1: Illustration of broadcast and brooding spawning modes used by deep-sea scleractinian stony branching corals.

4.1.3 Solitary cup corals (Order Scleractinia)

There have been no reproductive studies that have focused on solitary scleractinian cup corals from the New Zealand region, although genetic data suggest sexual reproduction and limited clonality for the cup coral *Desmophyllum dianthus* (Holland et al. 2020). Information from other regions suggests that species within this group exhibit different sexual systems (gonochoric or **hermaphroditic**), **reproductive modes** (i.e., spawning vs. brooding), **reproductive periodicity**, and larval development modes, even amongst **congeneric** species (Table 4-1). Within the family Caryophyllidae, *Caryophyllia smithii* and *Desmophyllum dianthus* are gonochoric and proposed to be seasonal broadcast spawners, based on studies from the NE Atlantic and Patagonian Fjords, respectively (Tranter et al., 1982; Feehan, 2016; Feehan et al. 2019). In contrast, individuals of *C. ambrosia*, *C. cornuformis*, and *C. seguenzae* from the NE Atlantic are hermaphroditic with apparent continuous or quasi-continuous reproduction (Waller et al., 2005). Evidence of planktotrophic larvae have only been observed for *C. smithii* (Tranter et al., 1982)*;* the other *Caryophyllia* species listed above are hypothesised to produce lecithotrophic larvae based on their large **oocyte** sizes. Currently one species, *C. inornata,* is known to be a gonochoric brooding species (Goffredo et al., 2012), though there are others under investigation in the Patagonian fjord region (Waller, pers comm.)

Within the genus *Flabellum*, reproductive modes vary across different habitats: *Flabellum* spp. collected from the NE Atlantic appear to spawn **gametes** (Mercier et al., 2011a; Waller and Tyler, 2011), while those on the continental shelf of the Western Antarctic Peninsula brood planulae (Waller et al., 2008). *Balanophyllia malouinensis* is another subantarctic solitary coral species that broods larvae (Pendleton et al., 2021), adding to the hypothesis that brooding may aid larval survival in Antarctic waters (Waller et al., 2008). At this time, little is known regarding the dispersal and settlement of brooded cup coral larvae, yet observations of the shallow-water temperate **azooxanthellate** species *Balanophyllia elegans* indicate that water flow and substrate availability

may be important settlement cues and contribute to the patchy distributions of this species (Altieri, 2003).

The difference in feeding modes between *Flabellum* spp. has been proposed in global studies as a potential reason for variation in reproductive periodicity (e.g., quasi-continuous versus seasonal spawners). For example, spawning of *F. angulare* in the NE Atlantic may be restricted to late August/September during seasonal phytoplankton blooms due to a more restricted diet of this species compared to the carnivorous or mixed diet of *F. alabastrum*, which reproduces continuously throughout the year (Waller and Tyler, 2011). **Asexual fission** has been observed in the species *Fungiacyathus marenzelleri* in the NE Atlantic, though at very low incidence, suggesting sexual reproduction was still dominant in this species (Waller et al., 2002), and overall, sexual reproduction appears to be the dominant reproductive mode of the stony cup coral group. **Fecundity** (i.e., reproductive potential, often measured as number of eggs/polyps) of cup corals is relatively high compared to other groups, but may decrease with depth and food availability (Waller et al., 2002; Flint et al., 2007; Mercier et al., 2011a; Waller and Tyler, 2011; Waller & Feehan, 2013).

4.1.4 Black corals (Order Antipatharia)

Knowledge of reproduction and larval biology of New Zealand antipatharians, black corals, is lacking for most species, although studies of the shallow-water Fiordland coral *Antipathella fiordensis* suggest this species is gonochoric (Miller, 1996; Parker et al., 1997), and produces lecithotrophic, weak swimming larvae with limited dispersal (Miller, 1997, 1998). *A. fiordensis*, and *A. wollastoni* from the Azores region in the Atlantic, and *Antipathes griggi* from Hawaii have each been hypothesised to have seasonal reproduction coinciding with increased sea surface temperatures during summer (Miller, 1996; Wagner et al., 2012b; Rakka et al., 2017).

Species within the *Antipathella* and *Antipathes* genera occur across broad depth ranges, though due to previous studies being conducted in shallower depths, it is unclear whether seasonality is common among individuals in deeper areas.

Reproductive investigations have been conducted for approximately 20% of the total number of described antipatharian species, although a majority of these are from individuals collected from shallow water regions (Wagner et al., 2012a). Of these, all species appear to be strictly gonochoric, with the exception of *Stichopathes saccula* (see reviews by Wagner et al., 2011, 2012a, and references therein). It should be noted, however, that nearly all previous studies of antipatharian reproductive modes have collected samples over a short term (i.e., < 1 year), and thus sequential hermaphroditism, where individuals change their sex, cannot be ruled out as a reproductive strategy (e.g., Wagner et al., 2012b). To date, there is no evidence of internal fertilisation occurring within antipatharian polyps; thus most species are hypothesised to spawn gametes (Wagner et al., 2012a). Compared to scleractinian species, polyp fecundity of antipatharian corals appears relatively low, potentially due to differences in oocyte size amongst these groups (Lauretta and Penchaszadeh, 2017). For further details regarding antipatharian reproduction, see the review by Wagner et al. (2012a), as in this report we have only summarised studies from the New Zealand region and those published after the review by Wagner et al. (2012a).

4.1.5 Gorgonian octocorals (Order Alcyonacea)

No specific New Zealand studies have been carried out for the gorgonian octocorals in this group, however from morphological studies many species of primnoid octocorals in the region have now been described as brooders (S. Soto de Matos-Pita, pers. comm.; Cairns, 2021), and some New Zealand primnoids have been described as gonochoric (Cairns, 2021).

Of the deep-sea gorgonians for which reproductive information exists globally, this group is almost entirely composed of gonochoric species (Simpson et al., 2005; Orejas et al., 2007; Kahng et al., 2011; Mercier and Hamel, 2011; Beazley and Kenchington, 2012; Feehan and Waller, 2015; Fountain et al., 2019; Waller et al., 2019).

The literature from other regions shows that deep-sea gorgonian species have varied reproductive modes, including continuous, quasi-continuous and seasonal spawning, and brooding of larvae. Apparent brooders include the primnoids *Fannyella rossii*, *F. spinosa*, and *Thouarella* sp. (Orejas et al., 2007), and the precious coral *Corallium rubrum* (Priori et al., 2013). Two other corallid precious corals, *Hemicorallium lauuense* and *Pleurocorallium secundum* are predicted to be quasi-continuous broadcast spawners, based on the simultaneous presence of oocytes and spermatocysts at various stages (Waller and Baco, 2007). Within the families Isididae and Plexauridae, and the genus *Primnoa* (family Primnoidae), broadcast spawning also appears to be the dominant reproductive mode (Mercier and Hamel, 2011; Beazley and Kenchington, 2012; Feehan and Waller, 2015; Fountain et al., 2019, Waller et al., 2019), though surface brooding on the epidermis of the polyps is suspected in *Primnoa pacifica* from the Gulf of Alaska (Waller et al., 2014).

Very little is known regarding the reproductive periodicity and larval biology of deep-sea gorgonians. Clear evidence of seasonal spawning has only been observed for the isidid *Keratoisis ornata* (Mercier and Hamel, 2011). Spawning by *K. ornata* colonies off the Eastern coast of Canada occurs in late summer/fall to coincide with increased seawater temperature and detritus deposition. The lack of evidence for seasonal reproduction in other gorgonians sampled from the NE Atlantic, such as *Acanella arbuscula* and *Primnoa resedaeformis*, could be due to limited temporal sampling and/or environmental differences, such as depth and food availability (Mercier and Hamel, 2011; Beazley and Kenchington, 2012). Additionally, species that appear to undergo continuous spawning or **gametogenesis** may still be influenced by environmental cues, and spawn on an opportunistic basis when resources are maximised (Mercier and Hamel, 2011). Ideally, to adequately determine species' reproductive modes and periodicity, samples need to be collected across a broad range of months.

There is a predicted relationship between oocyte size and reproductive mode in which brooders have larger oocytes than spawners, although this does not seem to hold true for gorgonians (Orejas et al., 2007). Based on their relatively large oocyte sizes, *A. arbuscula*, *K. ornata*, and *P. resedaeformis* are each hypothesised to produce lecithotrophic larvae (Mercier and Hamel, 2011; Beazley and Kenchington, 2012). It is currently unclear as to whether this is indeed the dominant feeding mode amongst deep-sea gorgonian larvae.

Polyp fecundity (often measured as the number of oocytes per polyp) of the gorgonian species included in this review is relatively low compared to the scleractinians (Table 4-1); though this difference may be offset by the large number of polyps in branched gorgonians, allowing for high total colony fecundity (Beazley and Kenchington, 2012). A positive correlation between coral colony size and polyp fecundity has been observed for some gorgonian species (Beazley and Kenchington, 2012; Priori et al., 2013), suggesting that larger colonies may contribute significantly more to localised reproduction. This correlation may weaken once coral colonies have reached a certain size (Fountain et al., 2019). Polyp fecundity can vary with polyp position along the colony, complicating estimates of total colony reproductive output. For example, some species show increased fecundity on the distal polyps compared to proximal polyps (e.g., *A. arbuscula;* Beazley and Kenchington, 2012), while others show the opposite trend with fecundity increasing towards the centre of the colony (e.g., several primnoid species; Orejas et al., 2007). Variation in fecundity has also been observed between individuals of the same species (Mercier and Hamel 2011; Fountain et al., 2019;

Waller et al., 2019), suggesting that: 1) environmental factors (in these cases geographical and depth differences) may be important to reproductive output, and 2) species fecundity estimates should be assessed regionally to account for potential intra-specific variation.

4.1.6 Hydrocorals (Order Anthoathecata, Family Stylasteridae)

There have been no published reproductive studies of New Zealand deep-sea hydrocorals, however morphological and molecular studies describing the New Zealand fauna have summarised their reproductive characteristics and larval behaviour. In a global diversity study of Stylasteridae, that included New Zealand species, Cairns (2011) provided a reproductive summary stating that stylasterid colonies are usually either male or female (i.e., gonochoric), rarely hermaphroditic, and always exhibit strong sexual dimorphism of the skeletal ampullae, which are visible cavities on the outside of the coral where the reproductive structures develop and that are often used to help identify species. Once the egg is fertilised, it grows to the advanced planular stage before it is released, after which it usually crawls away and settles a short distance from the parent. This produces rather limited distributions and high regional endemicity. A population genetics study of the endemic red coral *Errina novaezelandiae* indicated this hydrocoral species is gonochoric and hypothesised to brood planula larvae that crawl on the surface of adults before settling (Miller et al., 2004).

Stylasterid hydrocorals from the Aleutian Islands, Alaska, have also been determined to be gonochoric, with possible brooding in several species evidenced by the presence of planulae in individuals' ampullae (Brooke and Stone, 2007). Stylasterids are predicted to have limited dispersal due to their reproductive mode and larval behaviour, potentially resulting in the patchy spatial distributions frequently observed for these taxa (Miller et al., 2004).

Table 4-1: Reproductive characteristics of protected coral species summarised from the existing literature. Text denoted with an * refers to information that was inferred by the primary authors of the study, but not objectively proven as they may not have made any direct observations (i.e., is the most likely case). References in bold text refer to studies which were conducted within the New Zealand region. Blank cells signify no data available.

Group/species	Sexual system	Reproductive mode(s)	Larval nutritional mode	Larval behaviour	Fecundity	Mature size/age	Region	Reference
Stony corals (Order Scleractinia)								
Caryophyllidae								
Caryophyllia ambrosia	Cyclical hermaphrodite	Continuous broadcast spawner*	Lecithotrophic*		200-2,750 oocytes per polyp		NE Atlantic	Waller et al. (2005)
C. cornuformis	Cyclical hermaphrodite*	Broadcast spawner*	Lecithotrophic*				NE Atlantic	Waller et al. (2005)
C. seguenzae	Cyclical hermaphrodite	Quasi-continuous broadcast spawner*	Lecithotrophic*		52-940 oocytes per polyp		NE Atlantic	Waller et al. (2005)
C. smithii	Gonochoric	Seasonal broadcast spawner	Planktotrophic		Estimated as several thousand per polyp		NE Atlantic	Tranter et al. (1982)
Desmophyllum dianthus	Gonochoric	Seasonal broadcast spawner*			2,488 - 172,328 average oocytes per polyp	~82-150 mm^2 polyp area	Patagonian Fjords	Feehan (2016); Feehan et al. (2019)
Lophelia pertusa	Gonochoric		Lecithotrophic*		3,300 oocytes per cm^2 colony skeletal area	0.08 g polyp weight	NE Atlantic	Waller and Tyler (2005)
L. pertusa	Gonochoric	Broadcast spawner	Planktotrophic	Active swimmers			Trondheim Fjords, Norway	Larsson et al. (2014)
Goniocorella dumosa	Gonochoric	Seasonal broadcast spawner*; extratentacular budding; brooder (new observation this study)			480 ± 216 oocytes per polyp		Chatham Rise (SW Pacific)	**Burgess and Babcock (2005); NIWA this study**
Solenosmilia variabilis	Gonochoric	Seasonal broadcast spawner*; intratentacular budding			290 ± 144 oocytes per polyp		Chatham Rise (SW Pacific)	**Burgess and Babcock (2005)**

Group/species	Sexual system	Reproductive mode(s)	Larval nutritional mode	Larval behaviour	Fecundity	Mature size/age	Region	Reference
Dendrophyllidae								
Balanophyllia elegans		Brooder		Crawlers; short dispersal (~1 m)			NE Pacific	Altieri (2003)
B. malouensis		Brooder					Southern Ocean	Pendleton et al. (2021)
Enallopsammia rostrata	Gonochoric	Continuous broadcast spawner*			144 ± 96 oocytes per polyp		Chatham Rise (SW Pacific)	**Burgess and Babcock (2005)**
Flabellidae								
Flabellum alabastrum	Gonochoric	Quasi-continuous broadcast spawner*	Lecithotrophic*		maximum of 2,800 oocytes per polyp	0.247 g polyp wet weight	NE Atlantic	Waller and Tyler (2011)
F. angulare	Gonochoric	Seasonal or periodical* broadcast spawner	Lecithotrophic*		1,800-10,000 mature oocytes per female		NE Atlantic	Mercier et al. (2011)
F. angulare	Gonochoric	Seasonal or periodical broadcast spawner*	Lecithotrophic*		maximum of 550 oocytes per polyp	1.379 g polyp wet weight	NE Atlantic	Waller and Tyler (2011)
F. curvatum	Gonochoric	Brooder			1,618 ± 1,071 oocytes per polyp		Antarctica	Waller et al. (2008)
F. impensum	Gonochoric	Brooder			1,270 ± 884 oocytes per polyp		Antarctica	Waller et al. (2008)
F. thouarsii	Gonochoric	Brooder			2,412 ± 1,554 oocytes per polyp		Antarctica	Waller et al. (2008)
Fungiacyathidae								
Fungiacyathus marenzelleri	Gonochoric	Quasi-continuous broadcast spawner*; asexual fission	Lecithotrophic*		High (average 2,892 ± 44.4 oocytes per polyp)	10 mm polyp diameter	NE Atlantic	Waller et al. (2002)
F. marenzelleri	Gonochoric	Quasi-continuous broadcast spawner*	Lecithotrophic*		1,290 ± 407 SD oocytes per polyp		NE Pacific	Flint et al. (2007); Waller and Feehan (2013)
Oculinidae								
Madrepora oculata	Gonochoric	Seasonal broadcast spawner*					Chatham Rise (SW Pacific)	**Burgess and Babcock (2005)**
M. oculata	Gonochoric		Lecithotrophic*		7,680 oocytes per cm^2 colony skeletal area		NE Atlantic	Waller and Tyler (2005)

Group/species	Sexual system	Reproductive mode(s)	Larval nutritional mode	Larval behaviour	Fecundity	Mature size/age	Region	Reference
Oculina varicosa	Gonochoric	Seasonal broadcast spawner*			High (1,000-4,800 oocytes per cm2 colony skeletal area)		SE Florida Shelf	Brooke and Young (2003)
O. varicosa	Gonochoric		Lecithotrophic*	Active swimmers			NW Atlantic	Brooke and Young (2005)
Black Corals (Order Antipatharia)								
Antipathidae								
Antipathes griggi	Gonochoric	Seasonal spawner or pseudo-brooder*	Lecithotrophic*			~130 cm colony height	Hawaii	Wagner et al. (2012)
Myriopathidae								
Antipathella fiordensis	Gonochoric	Seasonal broadcast spawner*	Lecithotrophic*				Fiordland (NZ)	**Miller (1996)**
A. fiordensis	Gonochoric	Seasonal broadcast spawner*	Lecithotrophic*		~12-173.3 polyps per colony	70-105 cm colony height	Fiordland (NZ)	**Parker et al. (1997)**
A. wollastoni	Gonochoric	Seasonal broadcast spawner*			1-309 oocytes per polyp		Azores	Rakka et al. (2017)
Schizopathidae								
Dendrobathypathes grandis	Gonochoric	Broadcast spawner*	Lecithotrophic*		maximum of 9 oocytes per polyp		SW Atlantic	Lauretta and Penchaszadeh (2017)
Gorgonians (Order Alcyonacea)								
Chrysogorgiidae								
Metallogorgia melanotrichos	Gonochoric						NE Atlantic	Simpson et al. (2005)

Group/species	Sexual system	Reproductive mode(s)	Larval nutritional mode	Larval behaviour	Fecundity	Mature size/age	Region	Reference
Corallidae								
Corallium rubrum	Nearly all polyps and colonies gonochoric; one hermaphroditic polyp	Brooder			Average of 0.87 oocytes or planulae per polyp		NW Mediterranean	Priori et al. (2013)
Hemicorallium lauuense	Gonochoric*	Periodic or quasi-continuous spawner*					Hawaii	Waller and Baco (2007)
Pleurocorallium secundum	Gonochoric*	Periodic or quasi-continuous spawner*					Hawaii	Waller and Baco (2007)
Isididae								
Acanella arbuscula	Gonochoric	Broadcast spawner*	Lecithotrophic*		21 ± 17.5 oocytes per polyp	6.7-6.8 cm colony height	NE Atlantic	Beazley and Kenchington (2012)
Keratoisis ornata	Gonochoric	Seasonal broadcast spawner*	Lecithotrophic*		39 oocytes per polyp		Newfoundland and Labrador	Mercier and Hamel (2011)
Primnoidae								
Dasystenella acanthina	Gonochoric				1.2 ± 0.08 oocytes per polyp		Weddell Sea (Antarctica)	Orejas et al. (2007)
Fannyella rossii	Gonochoric	Brooder			1.5 ± 0.06 oocytes per polyp		Weddell Sea (Antarctica)	Orejas et al. (2007)
F. spinosa	Gonochoric	Brooder			1.4 ± 0.08 oocytes per polyp		Weddell Sea (Antarctica)	Orejas et al. (2007)
Primnoa notialis	Gonochoric	Broadcast spawner*			18 ± 4.51 oocytes per polyp		E Pacific	Feehan and Waller (2015)
P. pacifica	Gonochoric	Broadcast spawner*					E Pacific	Feehan and Waller (2015)
P. pacifica	Gonochoric	Broadcast spawner*			6.1-55.7 oocytes per polyp		Gulf of Alaska	Waller et al. (2014); 2019

Group/species	Sexual system	Reproductive mode(s)	Larval nutritional mode	Larval behaviour	Fecundity	Mature size/age	Region	Reference
P. resedaeformis	Gonochoric	Broadcast spawner*	Lecithotrophic*		84 oocytes per polyp (PRF); 9 mature oocytes per polyp (PRF)		Newfoundland and Labrador	Mercier and Hamel (2011)
P. resedaeformis	Gonochoric with one instance of hermaphroditism				16.5 ± 2.5 oocytes per polyp	7.6-19.8 years	Gulf of Maine	Fountain et al. (2019)
Thouarella sp.	Gonochoric	Brooder			1.1 ± 0.1 oocytes per polyp		Weddell Sea (Antarctica)	Orejas et al. (2007)
Plexauridae								
Paramuricea placomus	Gonochoric						NE Atlantic	Simpson et al. (2005)
P. placomus	Gonochoric				23.4 ± 4.3 oocytes per polyp	20.7-37 years	Gulf of Maine	Fountain et al. (2019)
S. beringi	Gonochoric	Broadcast spawner*			13.6 ± 2.85 oocytes per polyp		E Pacific	Feehan and Waller (2015)
S. kofoidi	Gonochoric	Broadcast spawner*			3 ±1.53 oocytes per polyp		E Pacific	Feehan and Waller (2015)
S. pacifica	Gonochoric	Broadcast spawner*			4.6 ± 2.06 oocytes per polyp		E Pacific	Feehan and Waller (2015)
S. simplex	Gonochoric	Broadcast spawner*			42.53 ± 9.82 oocytes per polyp		E Pacific	Feehan and Waller (2015)
S. spauldingi	Gonochoric	Broadcast spawner*					E Pacific	Feehan and Waller (2015)
S. torreyi	Gonochoric	Broadcast spawner*			8 ± 1.15 oocytes per polyp		E Pacific	Feehan and Waller (2015)
Hydrocorals (Family Stylasteridae)								
Crypthelia trophostega	Gonochoric	Brooder					Aleutian Islands (Alaska, USA)	Brooke and Stone (2007)

Group/species	Sexual system	Reproductive mode(s)	Larval nutritional mode	Larval behaviour	Fecundity	Mature size/age	Region	Reference
Cyclohelia lamellata	Gonochoric	Brooder					Aleutian Islands (Alaska, USA)	Brooke and Stone (2007)
Distichopora borealis	Gonochoric						Aleutian Islands (Alaska, USA)	Brooke and Stone (2007)
D. sp.	Gonochoric						Aleutian Islands (Alaska, USA)	Brooke and Stone (2007)
Errina novaezelandieae	Gonochoric	Brooder*		Crawlers*; short larval dispersal*			Fiordland (NZ)	**Miller et al. (2004)**
Errinopora nanneca	Gonochoric	Brooder					Aleutian Islands (Alaska, USA)	Brooke and Stone (2007)
E. pourtalesi	Gonochoric						Aleutian Islands (Alaska, USA)	Brooke and Stone (2007)
Stylaster brochi	Gonochoric						Aleutian Islands (Alaska, USA)	Brooke and Stone (2007)
S. campylecus	Gonochoric	Brooder					Aleutian Islands (Alaska, USA)	Brooke and Stone (2007)
S. cancellatus	Gonochoric						Aleutian Islands (Alaska, USA)	Brooke and Stone (2007)
S. verrillii	Gonochoric						Aleutian Islands (Alaska, USA)	Brooke and Stone (2007)
S. sp. 1 (*sensu* Brooke & Stone)	Gonochoric						Aleutian Islands (Alaska, USA)	Brooke and Stone (2007)
S. sp. 2 (*sensu* Brooke & Stone)	Gonochoric						Aleutian Islands (Alaska, USA)	Brooke and Stone (2007)

4.2 Examination of preserved samples

Results from a NIWA Invertebrate Collection database interrogation to recommend further species for reproductive studies are presented. From this, we found that there were numerous samples held in formalin, but several of these go back many years, likely impacting their usefulness for reproduction research. Long fixation periods can compromise the tissue, although it does depend on curation methods, e.g., how often the storage fluid may have been changed.

From the summary of corals in formalin, or previously fixed in formalin now held in ethanol, there were 73 scleractinian stony corals and 94 alcyonacean corals including gorgonian octocorals (Appendix 1). A sub-set of these has been compiled to indicate potential samples for a histological study, comprising protected corals collected since 2010 (Table 4-2 and Table 4-3).

Table 4-2: Protected coral samples and count of specimens stored in formalin previously, now stored in ethanol: stony corals by species. Stony coral species, jar count, and sample size. Details in column 3 are provided only for the more recent samples (2010 onwards). For additional sample details see Appendix 1. These samples have been fixed in formalin then transferred reasonably quickly post collection into 80% ethanol. The priority samples to be considered for a reproduction study are those highlighted (grey) (n= 22 sample jars). Note: the stony branching coral colonies have numerous individual polyps per colony, each one available for sectioning.

Scleractinian stony corals - cup and branching forms	Count (sample jar)	Total number of organisms within the subset of jars (year collected, if post 2010). The suggested study species are highlighted (n=19 cup corals plus numerous polyps of branching coral form)
Caryophyllidae (stony cup coral)	2	
Caryophyllia scobinosa	1	
C. profunda	5	14 (2018/2020)
C. diomedea	11	33 (2018)
Conotrochus brunneus	2	
Desmophyllum dianthus	**9**	**19 (2018)**
Goniocorella dumosa	**10**	**numerous (2019/2020)**
Labyrinthocyathus langae	1	16 (2018)
Solenosmilia variabilis	13	numerous (2014)
Stephanocyathus spiniger	1	
S. platypus	6	
Trochocyathus cepulla	2	
Vaughanella multipalifera	1	2 (2018)
Caryophyllia. unidentified	1	
Oculinidae (stony branching coral)		
Javania lamprotichum	1	
Madrepora oculate	6	
Dendrophyllidae (stony branching coral)		
Enallopsammia rostrata	**3**	**numerous (2020)**
Rhizangiidae (stony cup coral)		
?*Astrangia*	1	
Flabellidae (stony cup coral)		
Javania lamprotichum	1	1 (2018)
Polymyces wellsi	1	1 (2018)

Scleractinian stony corals - cup and branching forms	Count (sample jar)	Total number of organisms within the subset of jars (year collected, if post 2010). The suggested study species are highlighted (n=19 cup corals plus numerous polyps of branching coral form)
Flabellum spp.	1	1 (2018)
F. knoxi	1	2 (2018)
F. aotearoa	2	

Table 4-3: Protected coral samples and count of specimens stored in formalin previously, now stored in ethanol: Gorgonian octocorals. Gorgonian octocorals species, jar count, and sample size. Details in column 3 are provided only for the more recent samples (2010 onwards), that would be most appropriate for a reproduction study. For additional sample details *see* Appendix 2. These samples have been fixed in formalin then transferred reasonably quickly post collection to 80% ethanol. The priority samples to be considered, are those highlighted (grey) (n= 4 sample jars). Note: the coral colonies have numerous individual polyps per colony, each one available for sectioning). The endemic soft coral, not protected, also to be considered (n= 12 sample jars, 20 individuals sampled since 2010).

Alcyonacea gorgonian octocorals plus one endemic soft coral	Count (sample jar)	Number of organisms = numerous per jar from 4 colonies (year collected post 2010) The suggested study species are highlighted
Chrysogorgiidae (Golden corals)		
Iridogorgia spp.	1	(2010)
Isididae (Bamboo corals)	3	
Acanella	2	
Keratoisis glaesa	1	
K. tangentis	1	
K. spp	1	
Lepidisis spp	4	(2010/2011/2012)
Minuisis	1	(2009)
Paragorgiidae (Bubblegum corals)		
Paragorgia alisonae	3	
P. arborea	**2**	**(2010)**
Primnoidae (Sea fan, whip, bottlebrush)	2	
Arntzia gracilis	2	
Metafannyella moseleyi	1	
Parastenella spinosa	1	
Primnoa notialis	**2**	**(2010)**
Primnoella spp.	1	
Thouarella spp.	9	(2010/2020)
Taiaroiidae (Solitary octocoral)		
Taiaroa tauhou	12	20 (2010/2012)

While we limited our data extract and desktop exercise to two protected coral groups to focus in on what could be feasibly be achieved in a reproductive study, and to make use of coral samples that had been collected and stored in formalin in recent years, the NIWA Collection also holds some

samples of black coral and stylasterid hydrocorals in formalin. There are 12 black coral and 30 stylasterid hydrocorals held in the NIC, representing several species. All but three of these samples were collected post 2010, and therefore may be useful for histological examination.

4.3 Selection of key protected coral groups for reproduction studies

From the database mining exercise, a list of each coral sample and their corresponding metadata have been produced (see Appendices 1 and 2). These data provide some spatial (region and depth), information to help further research planning to investigate intra-specific variation in coral reproduction. The highlighted species in Table 4-2 & 4-3 (produced from the Appendices) are our recommended protected coral species list for a reproductive study. These are the scleractinian stony corals *Desmophyllum dianthus*, *Goniocorella dumosa*, and *Enallopsammia rostrata*, and the gorgonian octocorals *Paragorgia arborea*, and *Primnoa notialis.* The unique New Zealand endemic soft coral *Taiaroa tauhou* although not protected, may also be worth considering.

There are 19 stony cup coral *Desmophyllum dianthus* samples and numerous stony branching coral *Goniocorella dumosa* colonies comprising several polyps per colony that could be considered for further reproductive studies. All have been collected since 2010 and are currently in formalin. The *G. dumosa* samples are colony fragments with several polyps per colony available for histology. Around 20 polyps are present on half a fist size piece of coral fragment and so adequate numbers are readily available as we have many fragments. Burgess & Babcock (2005) examined 24 polyps per stony branching coral species. But this research covered only one time period (April 2001).

The *G. dumosa* numbers can be added to by using histology samples prepared as part of the NIWA's ROBES Programme. For ROBES, histological examination is being carried out to assess the impact of sediment on this coral species' internal tissue and organs, including reproductive organs, therefore there are slides available that we can examine from this research, Mobilia, submitted). While the samples have been prepared from organisms held in aquaria, they will be useful to obtain reproductive information for a specific time period, and thus increase the overall sample size.

From the gorgonian octocoral samples, there are 4 colonies collected in 2010 that have been identified as priority species to consider for a reproduction study: two bubblegum coral colonies (*Paragorgia arborea*), and two *Primnoa notialis* colonies. All colonies have several polyps per colony and were originally fixed in formalin and are now in ethanol. Expert advice has indicated that samples stored only in ethanol could also be useful to examine (Dr Rhian Waller, University of Maine, US). If ethanol fixed samples were also used, the sample numbers would be expanded significantly.

While protected corals are prioritised for a follow-up study, also to be considered is the endemic soft coral species *Taiaroa tauhou*. This deep-sea species displays solitary (i.e., not colonial) polyps, and is widespread on soft sediment on the Chatham Rise (Compton et al. 2013) and is the only extant Octocoral to grow as a solitary polyp, making it globally unique. There are 20 individuals collected since 2010 that would be available for the study. This is a unique collection and if reproductive material was found it may inform the sexual system and possibly reproductive strategies for this species.

5 Liaison with International coral experts

First author Di Tracey has liaised on a regular basis with coral reproduction expert Dr Rhian Waller, University of Maine, United States, prior to and since the inception of this Project. Dr Waller is a world renowned and well published expert on the reproductive strategies of deep-sea and Antarctic corals. Dr Waller's research is included in section 3.1.

The discussion that has taken place between Dr Waller and NIWA has focussed on a collaborative study involving students. The collaboration to date has included receiving advice on relevant recent literature, suitable species to research, methods, sample collection protocols for histology, and standardisation methods for sample maintenance.

We have discussed with Dr Waller the major goals of a potential future joint project to describe and quantify the baseline reproductive biology and output at both the colony and population level, for selected New Zealand protected corals. Potential project goals could incorporate three approaches:

1. Gross morphometrics of selected corals could be quantified from archived specimens by examining whole colonies and collecting data on polyp density, changes in polyp density across the colony, and the number of polyps per colony.

2. Histological analysis of preserved specimens could be utilized to collect data on male to female sex ratios, oocyte size distributions, fecundity per polyp, spermatocyst stage, and if possible, reproductive seasonality.

3. Archived video and images of coral communities could be analysed for: number of colonies, species distributions, and colony size.

Pairing gross morphometrics with histological analysis allows calculation of the reproductive output of entire coral colonies, and further coupling these data with video and image analysis and abundance estimates allows for scaling to whole population level by estimating the total reproductive output of local deep-sea coral populations. This innovative three-phase approach, which would be a first for deep-sea corals in New Zealand waters, would further develop the current concepts and methods used in this field.

The proposed research would occur during the 2021-2022 academic year.

6 Spawning event *Goniocorella dumosa*

Here we report on the deliverable for Objective 2 to describe the observed spawning event for *Goniocorella dumosa*. In September 2020, there was a breakthrough observation of coral larvae for the branching form of the deep-sea scleractinian species *G. dumosa*, one of the key habitat-forming, (but can be also bushy or clumped in appearance) stony corals located in the New Zealand region, as described in section 3.4. Spawning was observed in aquaria between September and November 2020.

6.1 Larval observations, behaviour and settlement

The larvae were orange in colour and primarily pear-shaped (Figure 6-1) although at times they were observed to be more spherical in shape. They were measured to be approximately 1.1 mm x 0.8 mm in size. Larvae had a formed mouth and were covered in small beating cilia.

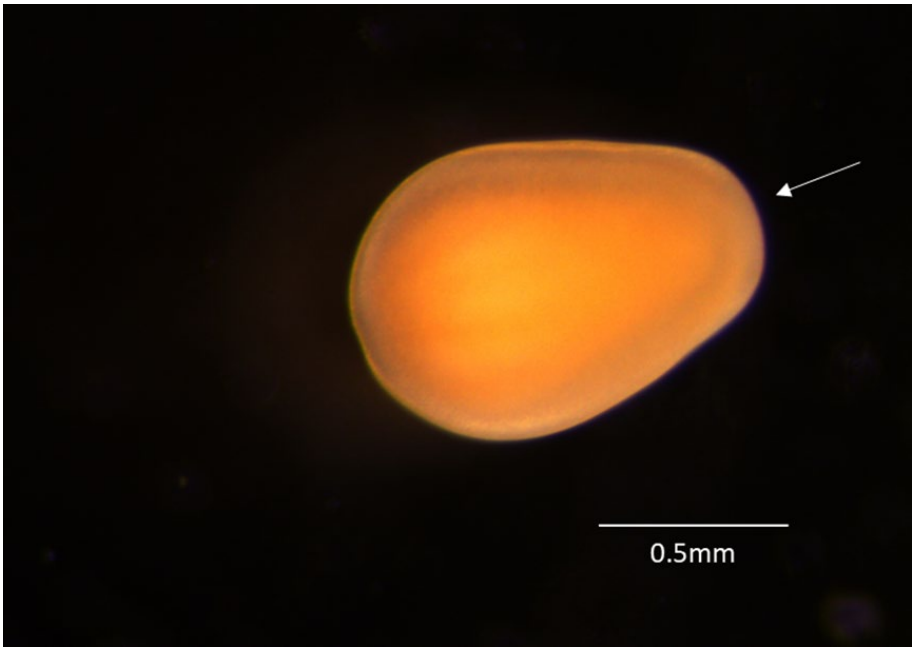

Figure 6-1: A swimming *G. dumosa* larvae. The formed mouth (not visible here) is on the right-hand side as indicated by the white arrow.

The *G. dumosa* larvae were observed swimming and were able to change speed and direction. At times they were observed moving near the bottom of the tank and at other times moving about mid-water within the tank. They did not swim constantly and were often observed "at rest". Note that the larvae were kept in small tanks in the dark and while observations were made daily, continuous observations were not feasible.

The *G. dumosa* larvae settled on a variety of substrates including plastic, silicone, and coral fragments Those that settled on plastic did not survive more than a few days, possibly due to this substrate medium not being suitable or from disturbance while trying to document their settlement. However, larvae did successfully settle and develop on silicone tubing and on fragments of the coral where no coenenchyme was present. Some larvae attempted to settle on the plastic floor of the holding tanks but while they started to develop **tentacles**, they did not successfully attach to this medium.

Figure 6-2 shows a newly settled *G. dumosa* on a fragment of coral branch (left image). The right image is the same individual 21 days post-settlement with tentacles (retracted when photographed) and a developing calcified structure.

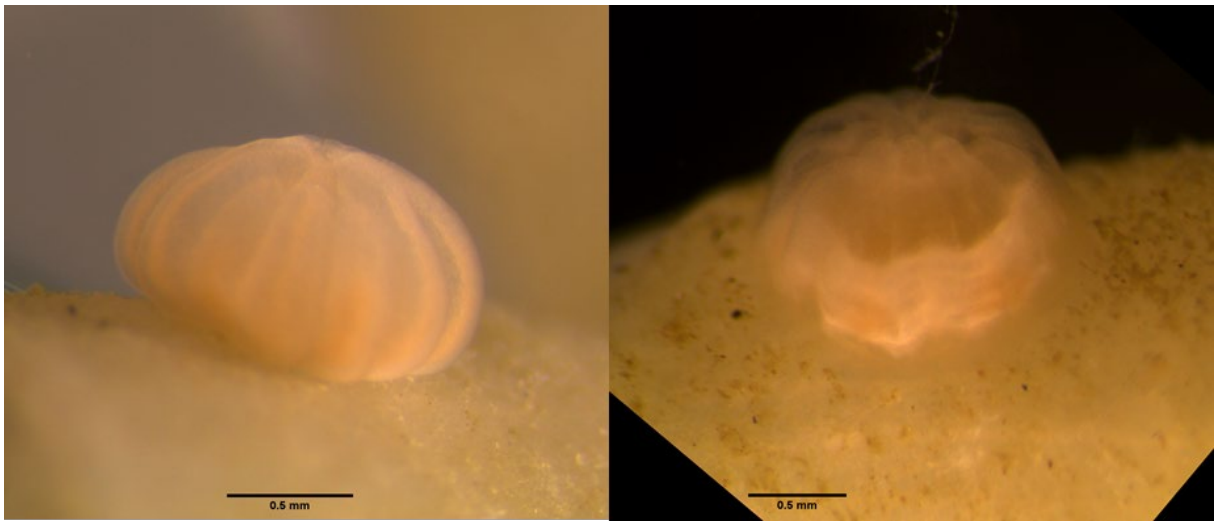

Figure 6-2: Settled *Goniocoralla dumosa* on a coral fragment. Left: newly settled and developing polyp. Right: 21 days post-settlement showing the polyp and the formation of the calcified skeleton. Scale bars are 0.5 mm.

6.2 Pelagic larval duration

Successful settlement (for those individuals where the larval release date was known) occurred between 2 and 8 days after larval release, with 5 out of the 6 larvae settling within 2 days (Figure 6-3). A seventh larvae was observed to settle between 1 and 3 days post larval-release.

A further six larvae b developing as if they had settled but failed to successfully attach to a hard substrate. This was observed 4 days post-larval release for 2 of the larvae. For the remaining 4 larvae it was not possible to determine an accurate larval release date, but they were estimated to be between 40 and 72 days since release. One *G. dumosa* larva was still alive and swimming 88 days post larval release and showed no signs of development.

The observed **Pelagic Larval Duration** (PLD) for successful settlement was just 2-8 days. However, with a larva still alive in the aquarium after 88 days it is possible the PLD may be longer.

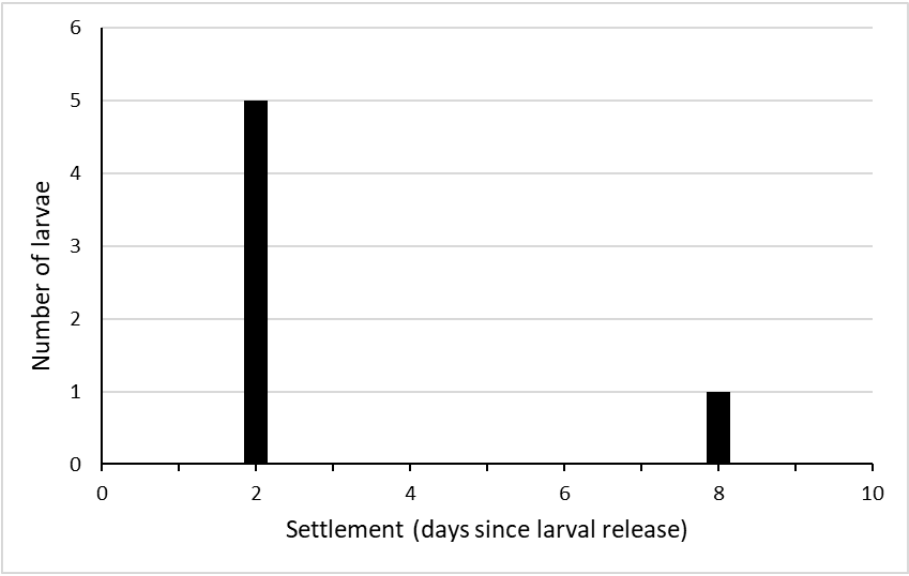

Figure 6-3: The observed time (days) to settlement of *Goniocorella dumosa* larvae which successfully settled onto hard substrates. Note that only those larvae for which the larval release date was known are included here.

6.2.1 Brooding

Further investigation using microscopy showed that this species is a brooder, with up to 10 mature larvae found in single mature polyps (Figure 6-4). Some larvae began swimming on release from the polyp during the dissection.

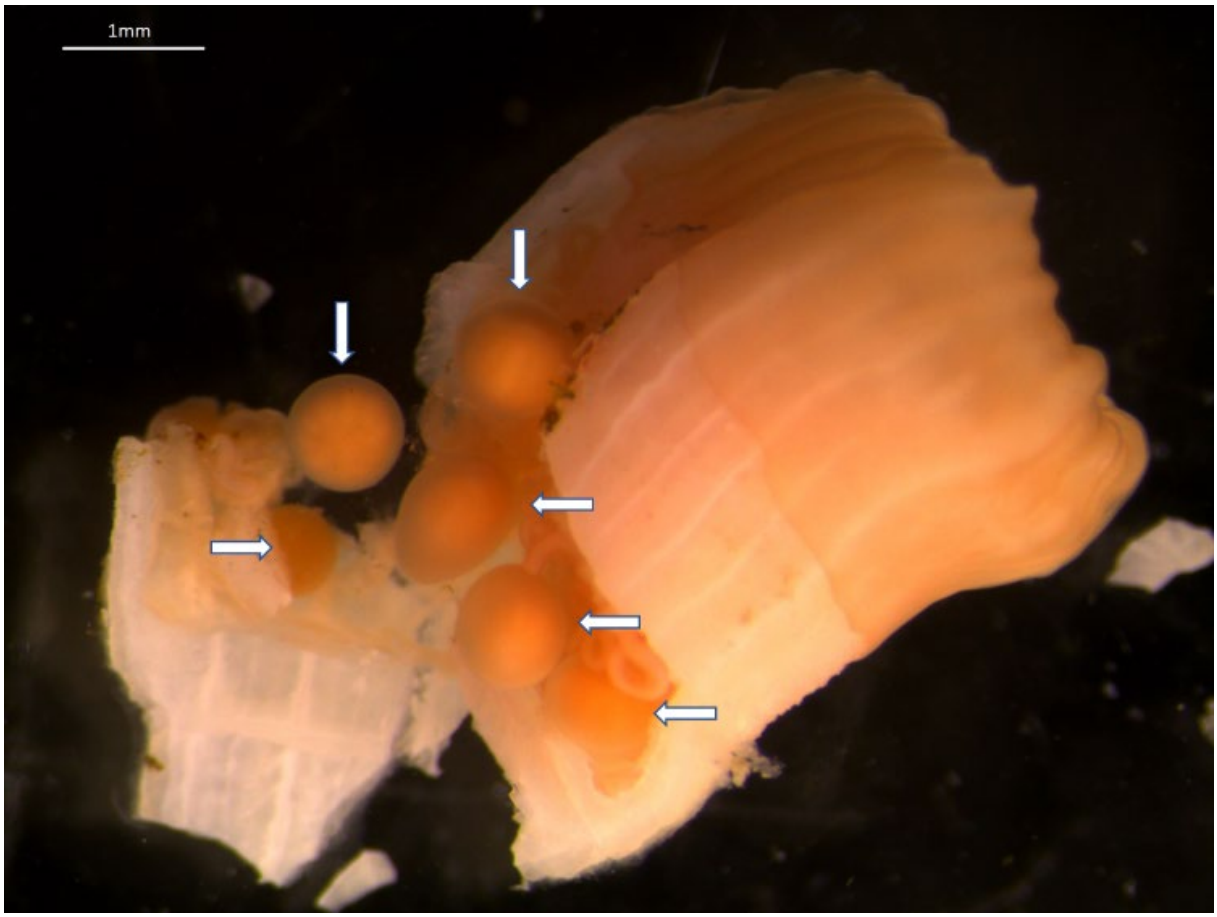

Figure 6-4: Brooding *G. dumosa* polyp. This polyp was dissected under a microscope and 10 larvae were located inside the mouth area. White arrows indicate the six larvae visible in this image.

6.2.2 Life-cycle of *Goniocorella dumosa*

A life-cycle schematic for *G. dumosa* is shown in Figure 6-5. A brooding polyp on a mature/adult colony of *G. dumosa* releases free-swimming larvae. After 2 to 8 days (and possibly longer, see above), the larvae settle onto a hard substrate. The formation of a calcified skeleton began after 2 to 3 weeks post settlement. After approximately 1.5 to 2 months, the small juvenile polyp has a formed skeleton and fully-formed tentacles.

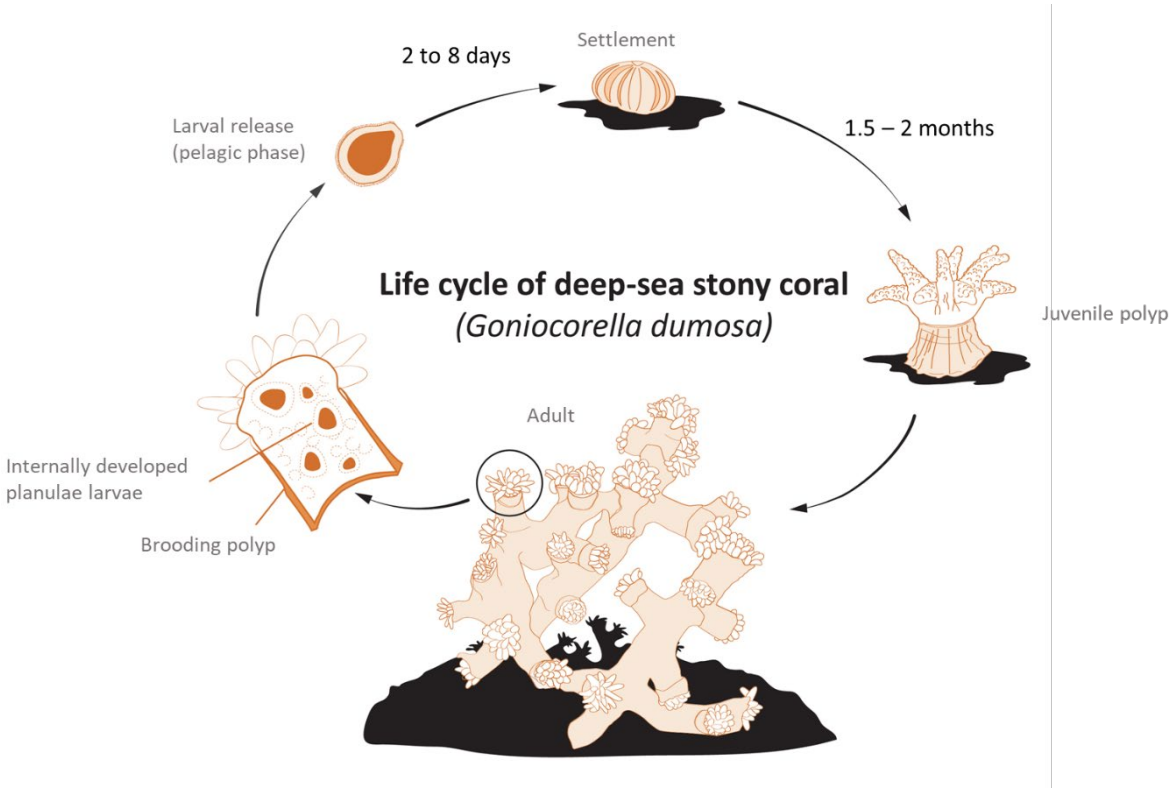

Figure 6-5: Life cycle schematic for *Goniocorella dumosa*. Showing the colony fragment with live polyps, a brooding polyp, the swimming larvae, settlement stage, and a juvenile polyp. Not to scale. The timeframes noted represent observations of successful settlement but note that one larva was still observed to be alive and swimming after 88 days.

6.3 Reproductive development

Histology slides of preserved specimens were examined to collect data on male to female sex ratios, oocyte size distributions, fecundity per polyp, spermatocyst stage, for the period June to September. Images of reproductive stages are shown in Figures 6.7 to 6.18.

Oocytes and spermatocysts were observed developing within the mesoglea of the mesenteries of parent polyps (Figure 6.7).

In females, few Type I oogonia or Type II immature oocytes (see Table 6-1, Figure 6.8) were observed. Most of the observed oocytes were Type III - developing (undergoing lipidogenesis) or Type IV - mature (full of large lipid globules) (Figure 6.9). Type V larvae were observed at various stages of development (Figures 6.10 – 6.14). The most advanced oocytes/larvae were generally positioned more basally within the mesenteries.

In males, sperm were observed developing in spermatocysts (Figures 6.16, 6.17). No Type I clusters of interstitial cells were observed. Spermatocyte forms Type II – IV (*see* Table 6-1, Figure 6.18) were common. Development of spermatocytes within a spermatocyst was synchronous. Development of spermatocysts within a mesentery or polyp was less so, the more basal spermatocysts in a mesentery showed the most advanced developmental state. No mature spermatozoa liberated from a spermatocyte were observed.

Table 6-1: Developmental stages of oocytes and spermatocytes (adapted from Burgess 2002).

Stage	Oocytes/Larvae	Spermaries
I	Oogonia: Enlarged interstitial cells, with large nuclei in mesoglea of mesenteries	Small clusters of interstitial cells
II	Immature Oocytes (previtellogenic): Accumulation of small amount of cytoplasm around nuclei	Spermatocytes smaller with small nuclei, number of cells within spermatocyst much larger
III	Oocytes undergoing Vitellogenesis: variable size, main period of vitellogenesis	Spermatocytes with little cytoplasm, developed flagella not evident, lumen usually present
IV	Vitellogenic Oocytes: full sized with indented nucleus migrating to edge of oocyte, large vitellogenin bodies fill the cytoplasm, cortical granular layer may be seen	Spermatozoa with fully developed flagella, ready to spawn
V	Brooding larvae of various stages of development	

Many of the histological sections exhibited significant scoring or areas of tissue absence, most likely due to inadequate decalcification or entrained sediment particles affecting the cutting of sections.

During the slide examination it was noted that all but one polyp was sectioned longitudinally, the remaining polyp was a tangential section (Figure 6.16), too close to the mouth to be of much use in the assessment of its reproductive state.

Observations were made of the numbers and stages of oocytes and larvae present in the sections observed. Dimensions of oocytes and larvae were measured where the section plane through the oocyte (contained a nucleus) or larvae (section appears to lie approximately mid-body) permitted. In males the numbers, stages and dimensions of spermatocysts (Waller & Tyler, 2002), were assessed.

Polyps from 24 coral fragments were prepared for histology for this work. Of those, thirteen of the fragments were female, eight were male, and three were of unknown observable sex due to their immaturity or the quality of the histological section. In all cases where the section included the main polyp as well as sexually maturing accessory polyps, all the polyps in the section were of the same sex.

6.3.1 Females

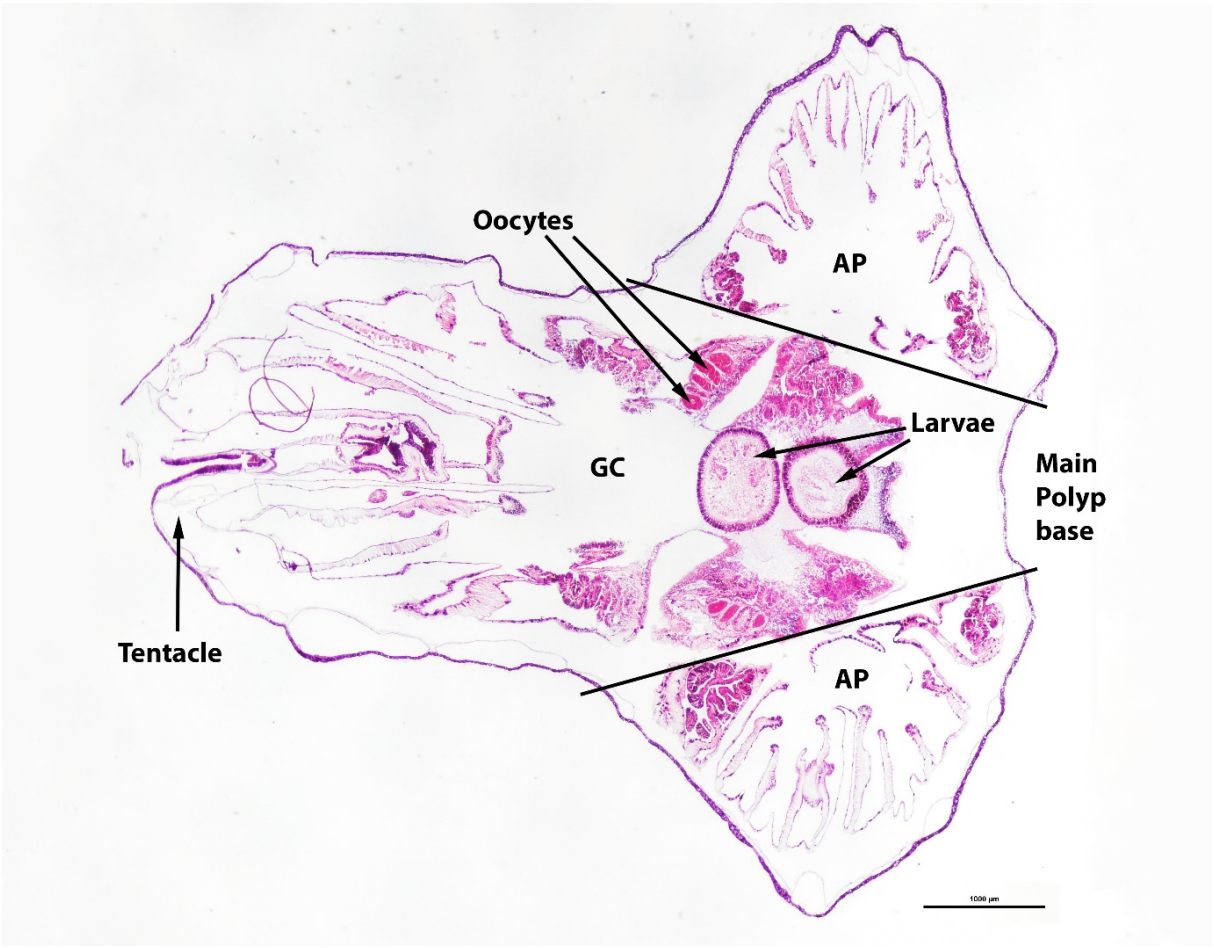

Figure 6-6: Central large primary polyp lying horizontally. Oral opening and tentacles to the left, base to the right, showing two mature larvae in the basal gut mesenteries. Clusters of Stage III oocytes can also be seen in the mesenteries of the gastrovascular cavity (GC). Accessory polyps (AP) extend upwards and downwards from this primary polyps' base, these two side polyps are immature, i.e. no gametogenic tissue was evident. Scale bar 1000 µm

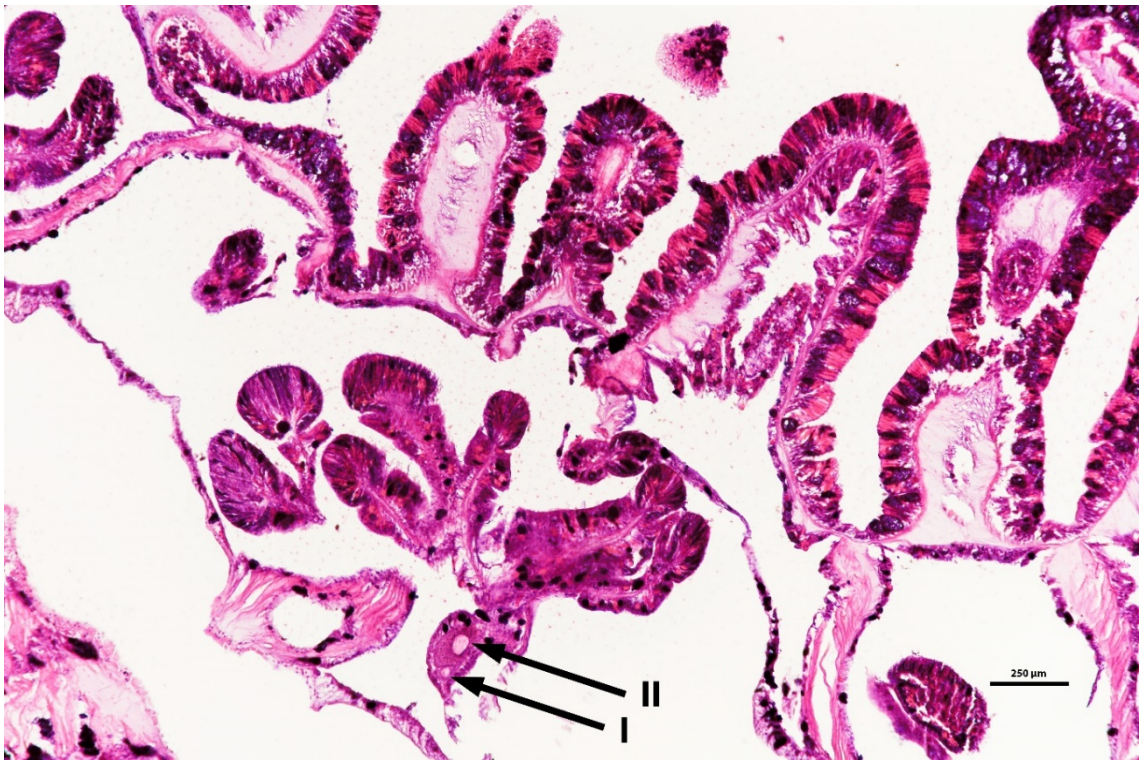

Figure 6-7: Stage I and II Oocyte . Stage I Oocyte (arrowed), displaying an enlarged interstitial cell with a large nucleus in the mesentery. A Stage II Oocyte sits adjacent to this, exhibiting accumulating cytoplasm. Scale bar 250 µm.

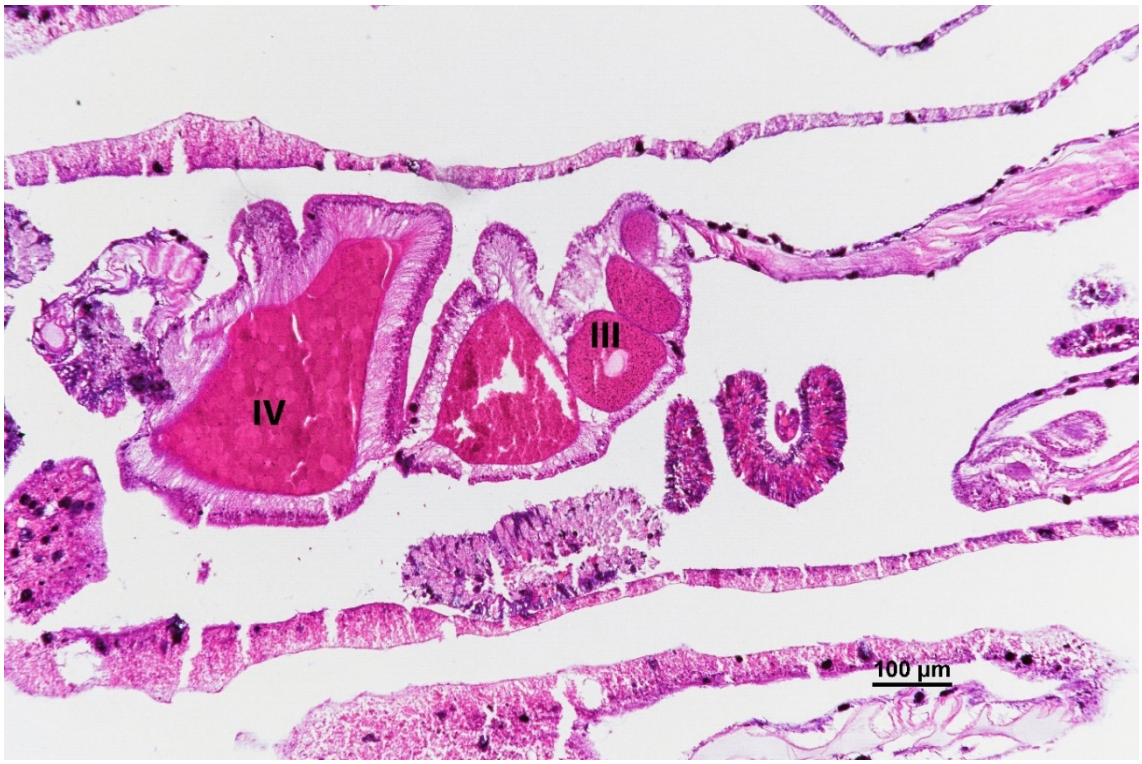

Figure 6-8: Stage III Oocytes. Stage III Oocytes displaying brightly pink staining vitellogenic bodies in the cytoplasm. The section cut has only gone through one of the four stage III oocytes nuclei. Stage IV Oocyte exhibiting mature globular vitellogenic bodies in the cytoplasm, this oocyte is a tangential section and has not intersected the nucleus. Scale bar 100 µm

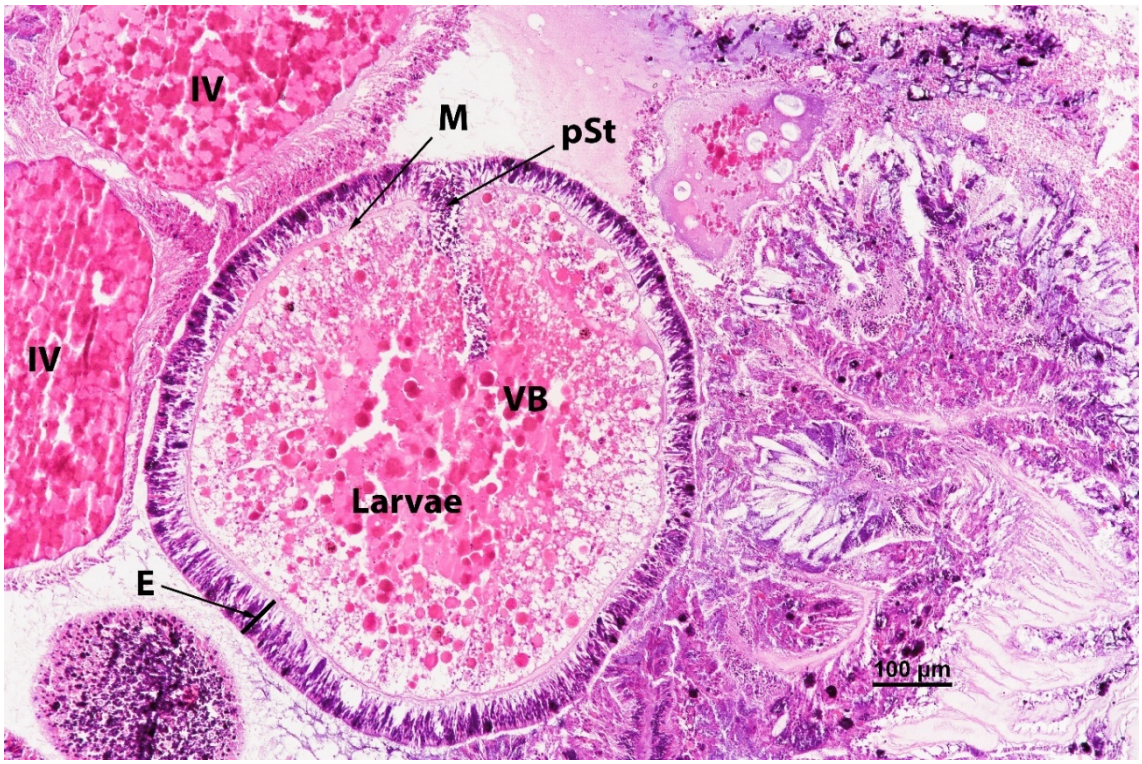

Figure 6-9: Stage IV Oocytes. Stage IV Oocytes displaying mature globular vitellogenic bodies. Stage 'V' Planula larva present. The larva visible in this image is obviously multicellular, already showing a high degree of cellular differentiation. Ectodermal layer (E) is well defined, sitting on a thin light pink staining mesogleal layer (M). The infolding ectoderm at the top of the larvae will form the stomodaeum (pSt), the future mouth. This end will be the oral pole. Vitellogenic bodies (VB) inside the larvae are still abundant but are being actively consumed (reducing in size and number). Scale bar 100 µm.

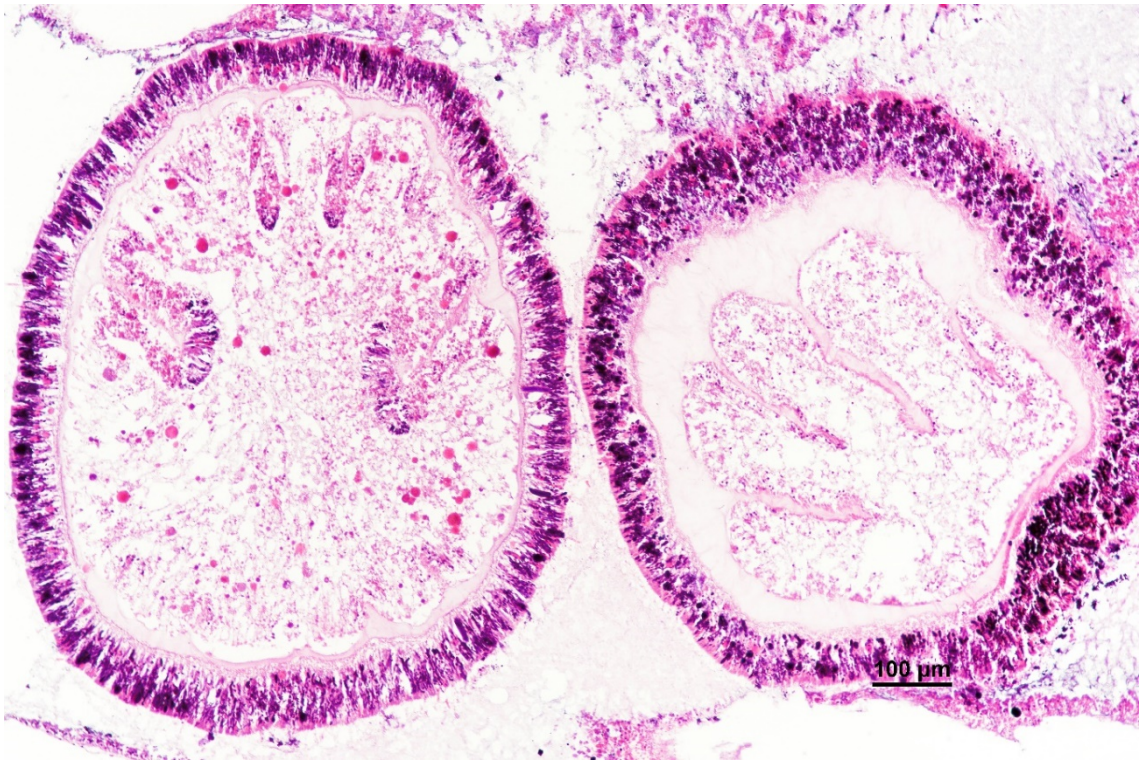

Figure 6-10: Two maturing larvae showing increased development of internal tissue. Larvae on the left is a near vertical section. Larvae on the right is a tangential section showing partitioning of the internal body space and development of mesenteries. Scale bar 100 µm.

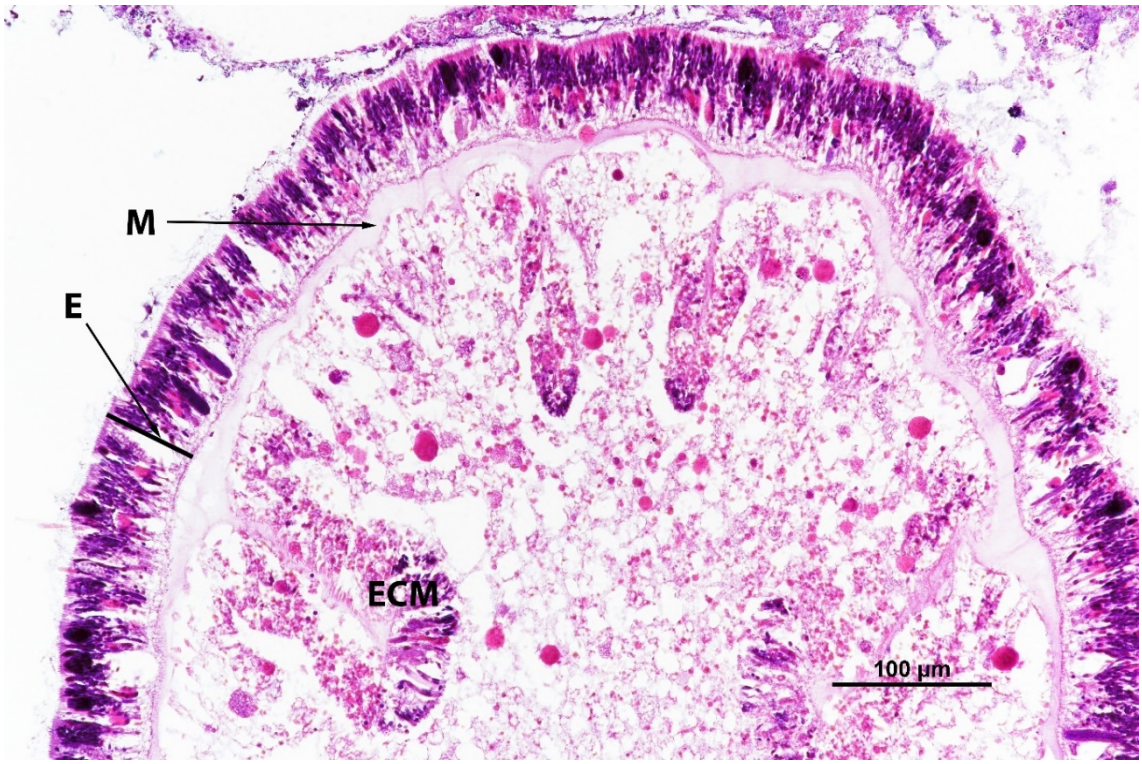

Figure 6-11: Showing detail of the developing larval body structures. Ectodermal layer (E) is well defined, sitting on a thin light pink staining mesogleal layer (M). Internal structure is starting to form, mesenteries arising from the Endodermal Cellular Mass (ECM). Scale bar 100 µm.

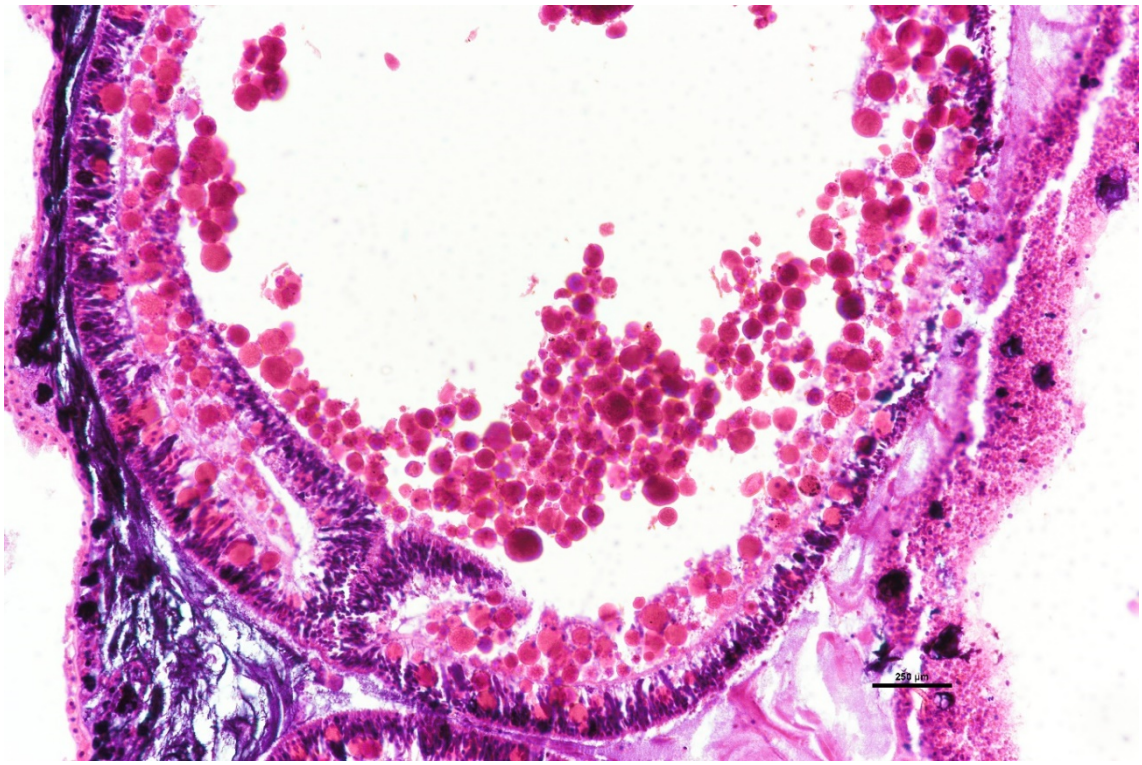

Figure 6-12: Close up section through a larva showing the developing stomodaeum which is open to the exterior. Scale bar 250 µm.

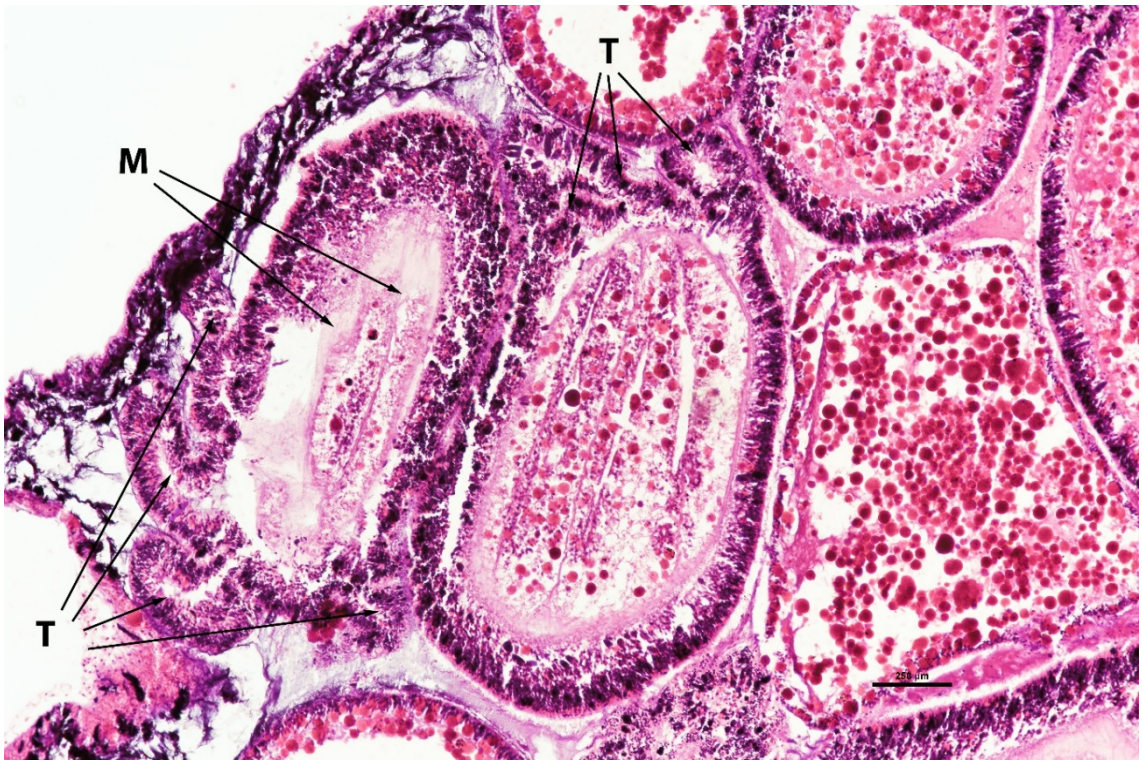

Figure 6-13: Two mature larvae exhibiting longitudinal internal body segmentation through the development of the mesenteries (M). These two larvae appear to show development of feeding tentacles (T) extending out from the oral end of the larvae. This shows larvae displaying a high degree of development prior to liberation from the parent polyp. Scale bar 250 µm.

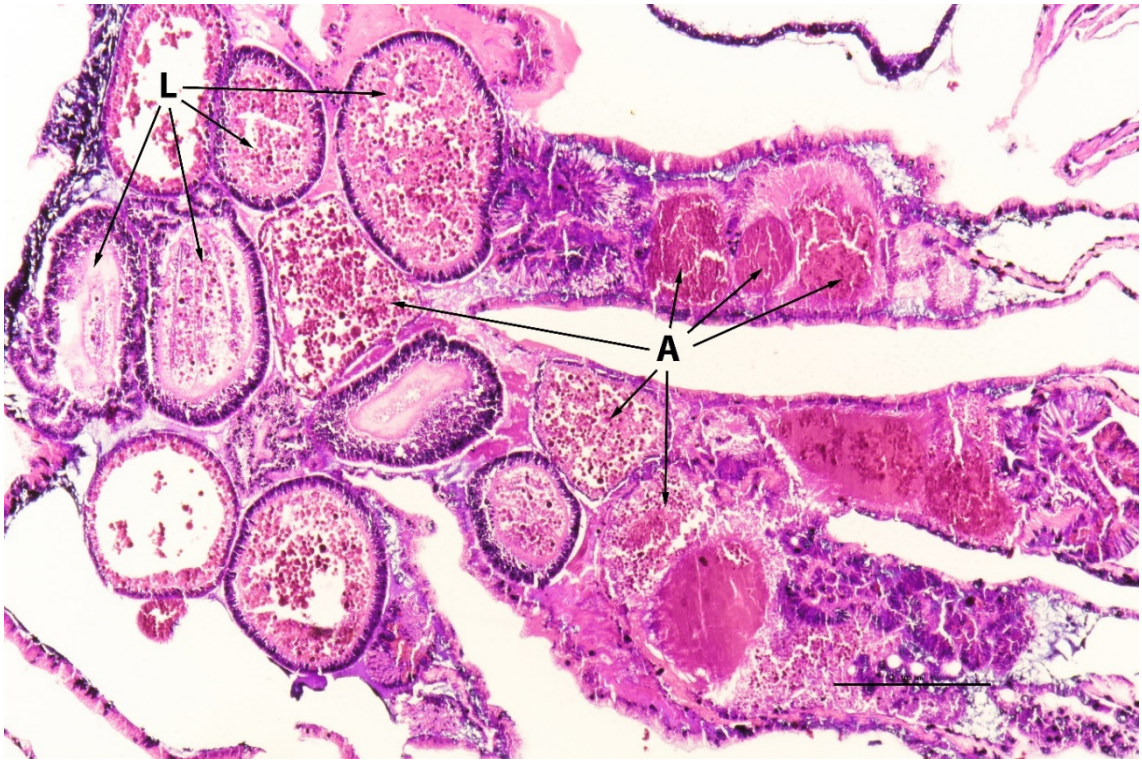

Figure 6-14: Polyp section showing a number of larvae (L) and numerous atretic masses (A) of degrading oocytes. Detail of two of the larvae are in Figure 6.8. Scale bar 500 µm.

6.3.2 Males

No obvious Stage I interstitial cells or Stage II spermatocytes were observed in the histological sections.

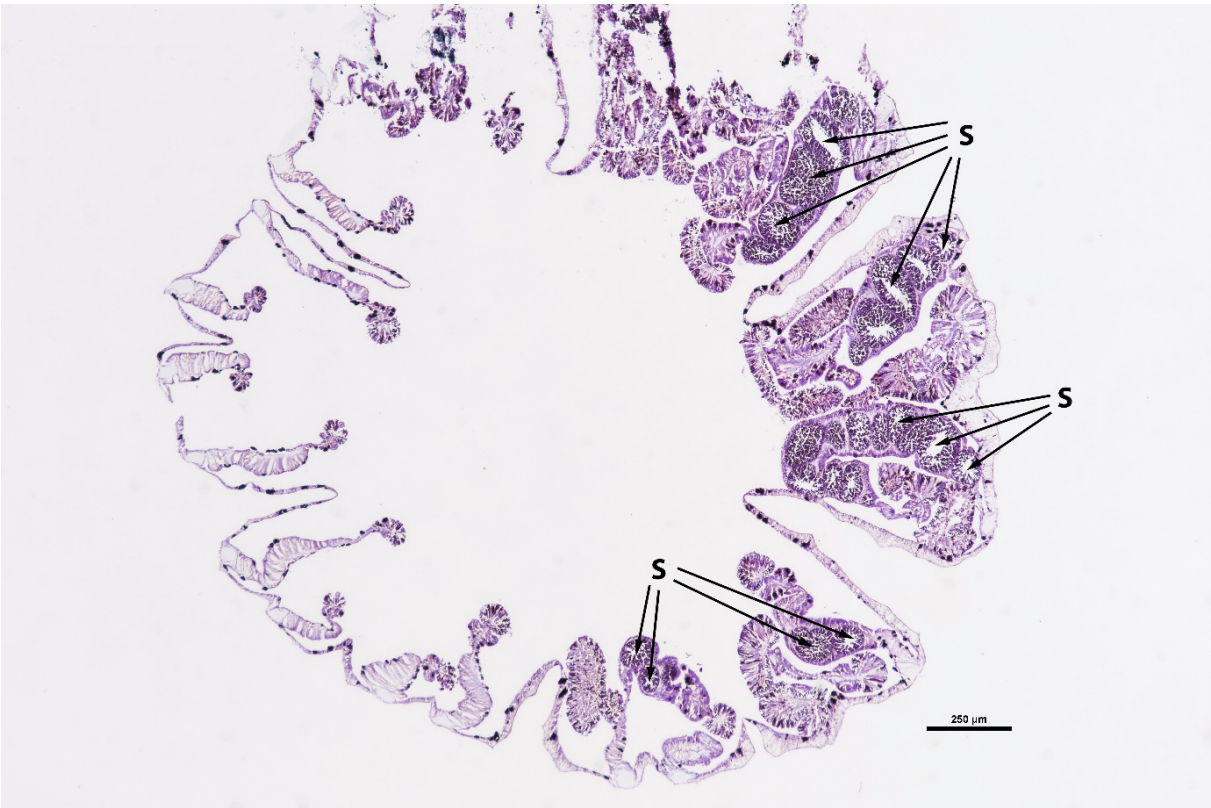

Figure 6-15: Tangential section across a male polyp showing clusters of spermatocysts (S) embedded in the mesenteries. Spermatocysts are visible in five adjacent complete mesenteries, so it is likely that all complete mesenteries would contain gametogenic tissue. This was the only cross section through a polyp prepared for this piece of work, due to its tangential plane half of the section was intersected too close to the oral aperture to see if all complete mesenteries contained gametes. Scale bar 250 µm.

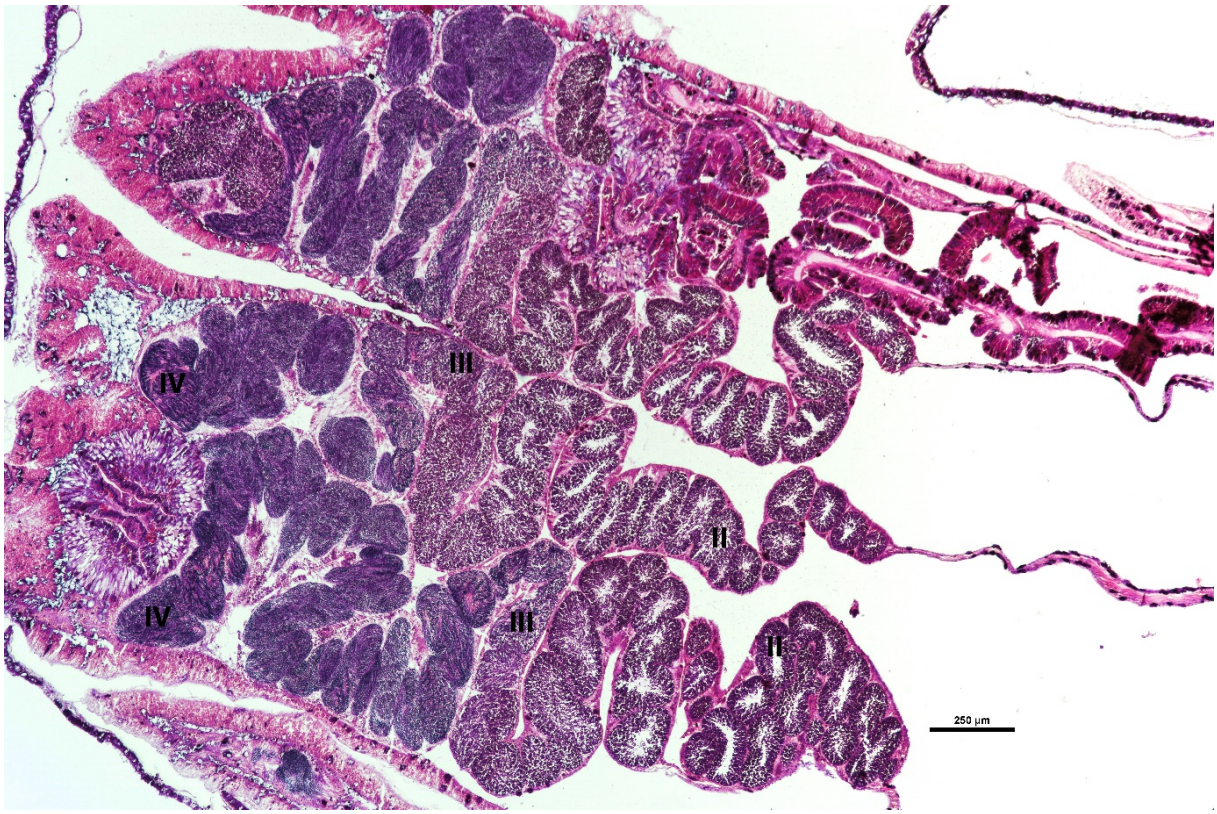

Figure 6-16: Basal section of male polyp showing various stages of spermatocyst development (II, III, IV). The spermatocysts of mature spermatozoa are sited most basally in the polyp mesenteries. Scale bar 250 µm.

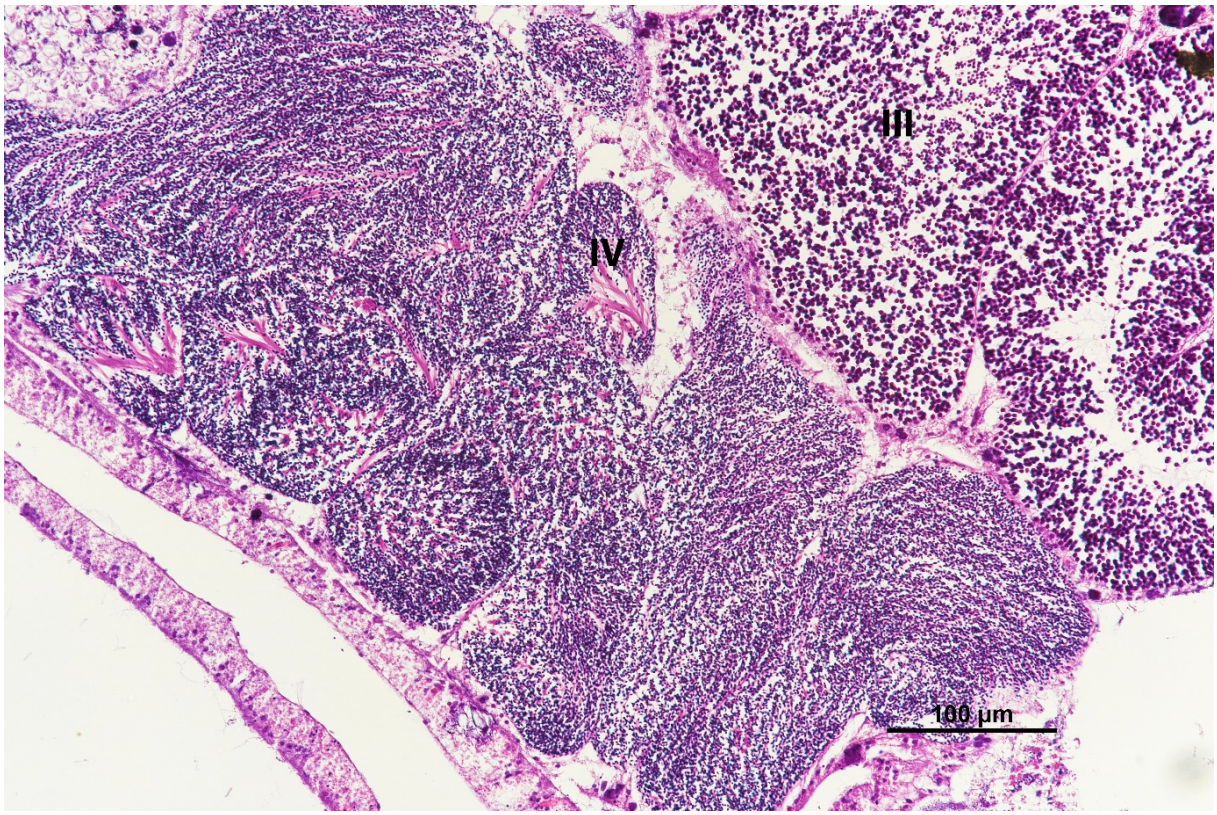

Figure 6-17: Detail of male spermatocytes and mature spermatozoa. Type III maturing spermatocytes show the start of the development of the flagellum, their nucleus is condensed and the cytoplasm is greatly reduced. Type IV mature flagellated spermatozoa. The flagella are oriented towards the centre of the spermatocyst within the lumen. Nuclei are extremely condensed and cytoplasm is minimal. Scale bar 100 µm.

6.3.3 Counts and fecundity estimates

Oocytes, larvae and spermatocysts visible in the clearest histological section on each slide were counted and measured. This gives us a minimum fecundity for each polyp. Serial levels were not taken for histology through each polyp and a significant proportion of oocytes would have been missed, so the total fecundity for a given polyp could not be assessed. The total fecundity per polyp would be well in excess of the numbers presented here. Twenty female polyps were observed exhibiting 2-84 oocytes and larvae per section (mean 12.40 standard deviation 17.69). A number of immature accessory polyps that did not exhibit any gametogenic tissue were not included in this data.

Intact oocytes, larvae and spermatocysts were measured across their longest axis and the axis perpendicular to this from histological sections. The mean of these two measurements was used to generate standardised sizes of the oocytes, larvae and spermatocysts as they are generally not round in cross section (Table 6-2). A temporal study would help clarify the timings of development between oocyte and spermatocyte stages.

Table 6-2: Oocyte, larvae and spermatocyst sizes by stage.

Sex	Stage	Count of oocytes / spermatocysts by stage	Minimum size µm	Maximum size µm	mean size µm	Standard deviation
Female	1	2	45	94	69	35.00
Female	2	11	63	211	117	46.92
Female	3	43	139	538	269	87.14
Female	4	25	428	931	668	139.47
Female	5	19	596	1220	904	157.81
Male	2	42	88	380	235	84.27
Male	3	137	116	545	234	97.83
Male	4	33	131	488	274	75.94

7 Summary and conclusions

The review of recent research in this field has highlighted that there remain several knowledge gaps for reproductive and dispersal capacity for deep-sea protected corals in the New Zealand region. Additionally, little information is available on larval motility, behaviour and duration. Often a corals reproductive mode has been inferred from data on related species, so recent advances in global research and reviews aid our understanding of the reproductive modes for various deep-sea corals.

7.1 Review

We know from an opportunistic in-aquaria observation that there is some reproductive variability within the scleractinian stony branching coral groups, where for one species the reproductive mode is brooding, while the others are still considered broadcast spawners. The *Flabellum* cup coral, a commonly occurring genus in the New Zealand region, was recently observed in the NE Atlantic to spawn gametes while those from the continental shelf off the Western Antarctic Peninsula brood planulae. A little more is now known about reproductive periodicity, and this factor needs to be considered for our region.

The previously considered strictly gonochoric black coral group has at least one exception overseas, with the possibility also of sequential hermaphroditism occurring . There is some seasonal spawning or pseudo-brooding in this group, e.g., surface brooding or where planulae released early from colonies without signs of internal brooding). It is worth noting that compared to the scleractinian species, polyp fecundity of antipatharian corals appears relatively low.

Gorgonian species have varied reproductive modes, and several recent examples show that many are brooders. Temporal sampling limitations and/or environmental factors, such as depth and food availability are increasingly affecting our conclusions of reproductive modes and cues.

Spawning can be periodic or quasi- continuous, and those species that show continuous spawning or gametogenesis may nevertheless be influenced by environmental cues, thus spawning on an opportunistic basis when resources are maximised. In a recent study the reproductive mode for plexaurid octocorals was only inferred by the authors and not directly observed, highlighting a knowledge gap for this understudied octocoral group.

Stylasterid hydrocorals continue to be regarded as brooders, based on observations of the advanced larval or planular stage seen to crawl away and settle a short distance from the parent. This produces rather limited distributions and high regional endemicity, an important consideration for managing impacts.

7.2 Spawning event

Larvae from a spawning event have not been observed before for any deep-sea coral in New Zealand waters. Indeed, spawning events have only been observed for a handful of deep-sea stony corals globally (Waller 2005), and unusually not as yet for the very well-studied species *Lophelia pertusa* (Larsson et al. 2014).

As described in section 3.1, previous New Zealand work indicated *G. dumosa* were broadcast spawners and that fertilisation was likely to occur in late April or May (Burgess & Babcock 2005). *G. dumosa* are now known to be gonochoric brooders and were observed to release planula larvae between September and November in aquaria. The larvae had a formed mouth and were observed swimming. Settlement after release was relatively quick, with a number of larvae settling within 2 days. Successful settlement occurred on both coral branches and silicone tubing, where they developed a calcified skeleton and feeding tentacles.

Until this recent observation, there was no information on larval development for any deep-sea coral species in New Zealand waters. No information existed on fecundity, larval behaviour, length of their larval phase prior to settlement, or post-settlement growth and development. Already, this serendipitous observation has changed our understanding of the reproductive and dispersal processes of this coral species.

The more limited dispersal potential of brooders, compared to broadcast spawners, has significant implications for population connectivity, and for the potential recovery of this species from human induced disturbance activities. This in turn should influence the management and protection of *G. dumosa* habitat within the region**.**

7.3 Histology of *Goniocorella dumosa*

Histological sections through the polyps of *Goniocorella dumosa* specimens show that they are gonochoric viviparous brooders, i.e., single sexed with internal fertilisation of gametes. The histology observations confirm our in -aquaria and microscopic observations. In all cases where the section included the main polyp as well as sexually maturing accessory polyps, all the polyps in the section were of the same sex. This indicates individual colonies of this coral are single sexed.

The limited numbers of polyps available for observation in this histological study, (n = 24), has not enabled us to present an accurate assessment of the sex ratios of the examined colony samples. The observed ratio of thirteen females to eight males indicates that the natural population sex ratio could be -relatively evenly heterogeneous.

Both males and females exhibited gametogenic material at a range of developmental stages within individual polyps, from reasonably immature to fully mature. This suggests the reproductive strategy for *G. dumosa* is likely to lie somewhere along the spectrum from serial spawner to continuous spawner. If instead these corals only utilised single batch spawning as a reproductive strategy, we would have seen all the maturing oocytes/larvae at a similar stage of development. This was not the case. As all the material for this study came from a narrow timeframe it is not possible to ascertain whether this coral species serially spawns over a protracted period or spawns more continuously throughout the year. The males observed in this study showed reasonable synchronicity between individuals indicating that the most likely reproductive strategy employed is serial spawner over a protracted 'season' of the year. Burgess (2002) observed mature Type IV oocytes in samples collected in April. Our samples presented Type II to Type IV oocytes and mature larvae in samples from August to September. It is likely that in this species in the New Zealand region oocyte maturation occurs over the Austral autumn/winter with spawning occurring late winter to early spring. This histological evidence ties in with experimental observations of spawning from September to November 2020.

The total fecundity per polyp could not be ascertained from this limited data set of histology preparations. Usually only one to three or four level sections were taken through a polyp at an unknown distance apart. Only a few septa were viewable in any given section, so no observations could be made concerning the number of septa in a polyp that are involved in gametogenesis, this observation would have helped to characterise the total fecundity of the polyp. It is likely that all complete mesenteries would contain gametes. From the sections available we could see that polyps produce an observed minimum of 2-84 oocytes and larvae per polyp, the total counts from these polyps would likely have been significantly in excess of this. Burgess (2002) estimated, from observed serial level sections through entire polyps, a total fecundity of 480 oocytes per polyp in a breeding season. This compares favourably with our findings from this study.

Oocytes and larvae observed in this study ranged from Type I (immature oogonia) to Type V (fully mature) larvae. The majority of oocytes observed were Types III and IV (maturing to mature oocytes) the larvae observed ranged from relatively undifferentiated planula larvae to well advanced larvae with a high degree of tissue development. Our observed Type I oocytes ranged from 45-94 µm, Type II oocytes from 63-211 µm, Type III from 139-538 µm, and Type IV oocytes from 428-931 µm. The larvae ranged in size from 596-1220 µm.

It is unknown whether Type II oocytes observed in histological section of polyps from August to September would have continued to mature and spawn that season or if they would have been held by the parent polyp in a partially matured state for final vitellogenesis and maturation the following spawning season.

Samples for this study were collected from the Chatham Rise at approximately 400 m in June 2019 and held in aquaria, samples for histology were taken in August and September 2020. Burgess (2002) utilised samples collected from the Chatham Rise from deeper waters, 890-1130 m in April 2001. All oocyctes observed in the Burgess study were 'Type IV' mature oocytes containing large yolk granules, with a maximum oocyte diameter of 135 µm. This is smaller than our observed Type IV oocytes. The Type IV oocytes we observed appeared to exhibit larger vitellogenic bodies in the cytoplasm, so we may be seeing a more advanced stage in the oocyte development than Burgess observed in her work. Our samples were also obtained from a much shallower water depth, approximately 400m, and then reared in aquaria for months, as opposed to the Burgess samples which were collected from 890-1130 m water depth. It is possible that our samples developed in a more nutrient rich environment enabling the oocytes to produce more and larger vitellogenic bodies, resulting in larger oocytes at a given stage.

We observed that the fertilised gametes are retained within the mesenteries until the stomodaeum, or oral opening, is well developed. Well advanced larvae still retained moderate reserves of lipid globules which would sustain the larvae, post-release, until the larvae can settle and develop fully functioning feeding apparatus. It is possible that larvae observed during their free-swimming phase may be able to actively feed to a limited extent, given that they had a formed mouth and that one larva was observed swimming in the water column for 88 days.

Some of the histological sections contained larvae of an advanced state with apparent development of feeding tentacles extending out from the oral end of the larvae, these larvae were still retained within the mesenteries of the parent polyp. It is possible these larvae have been retained unnaturally long by the parent polyp, pending unsatisfactory spawning cues presented in the aquarium environment, resulting in larvae that are exhibiting "anticipatory development of post-larval structures when metamorphosis is delayed" (Gleason and Hofmann 2011).

Some of the sections observed contained significant numbers of oocytes undergoing a process of atresia, as the non-viable oocytes are being re-absorbed into the parent polyp's tissues. The large number of atretic masses is potentially the result of the coral not finding the aquaria environment particularly suitable for gametogenesis, spawning events and larval development.

8 Recommendations

We recommend that reproductive studies be prioritised for the following species

- the scleractinian stony coral species *Desmophyllum dianthus*, *Goniocorella dumosa*, *Enallopsammia rostrata*
- gorgonian octocoral species *Paragorgia arborea*, and *Primnoa notialis*.

The research will help address some of the knowledge gaps for the coral groups in the New Zealand region. It is clear that some groups exhibit a lot more variability in coral reproductive mode and this information helps support the species-specific studies we recommend.

The species that have been prioritised for future research scored medium to high in the summary of Productivity and Susceptibility scores in the pilot risk assessment by Clark et al., (2014). The overall risk value and ranking for productivity specifically was high for the black corals, the black coral *Bathypathes*, and the bubblegum coral *Pargaorgia spp*., and medium for all scleractinian branching stony corals and for the gorgonian octocoral *Primnoa* spp. The risk assessment along with the empirical data listing available samples currently held in the NIC, particularly samples that were recently collected (post 2010) and appropriately preserved (formalin fixed then transferred to ethanol), have helped guide the recommendations in this report.

We also recommend that:

- Future research includes a meta-analysis of global literature on the reproduction of deep-sea corals. This exercise could determine regional, spatial (both by depth and latitude), and/or taxonomic patterns in corals sexual systems, reproductive strategies, oocyte size, fecundity estimates, and their larval biology.
- Future research funding could also prioritise rearing coral in-aquaria. It is challenging and costly to study the spawning and larval behaviour of deep-sea taxa *in situ* but successful and cost-effective in-aquaria research that has kept two species of stony branching corals alive in for extended periods (almost 2 years) has shown us that such experiments are feasible and can help determine spawning triggers, larval duration, and settlement preferences – information critical to developing species dispersal models.
- The examination of preserved *G. dumosa* colonies to identify the presence of any newly settled/calcified juveniles is recommended as this could indicate the likely timing/seasonality of larval release and settlement *in-situ* on the Chatham Rise. This would be in addition to the sample preparation work proposed to be carried out on the samples held in formalin. Further histological examination of existing specimens held in the National Invertebrate Collection could help to confirm the reproductive strategy utilised by this species of coral. It could also help to clarify the timing of the spawning season and help to get more realistic estimates of the fecundity of the polyps, whole colonies and coral reefs. This could underpin productivity parameters in future risk assessments.
- Analysis of specimens from around the New Zealand region would show if the reproductive strategy utilised by this coral was the same and timed similarly throughout the region.

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Appendix 1

Catalogue number, species name, sample position and depth data, sample count, and preservation method (ORIG = originally), data for Scleractinian stony coral samples selected from NIWA Invertebrate Collection Specify database *niwainvert* that have been or are currently preserved in formalin. From this list, samples collected post 2010 have been selected as recommended species for a reproduction study (see Table 4-2).

NIWA Invertebrate Collection Catalogue No.	Species name Family	Genus	species	Station code	Sample date	Position Latitude (S)	Longitude (all East unless shown as west=W)	Sample depth start (m)	finish (m)	Sample count	Preservation type
148161	Caryophylliidae	*Desmophyllum*	*dianthus*	TAN2009/80	19/08/2020	44.136166	174.7211666 W	640	622	11	Formalin
41990	Scleractinia (to Order only)			TAN0413/77	12/11/2004	37.47100067	177.2148285	180	177	1	Ethanol - orig formalin
127388	Caryophylliidae			Z10799	23/05/2001	35.73749924	178.4963379	365	202	1	Ethanol - orig formalin
127395	Caryophylliidae			TAN0107/323	24/05/2001	36.1456667	178.2016667	924	712	1	Ethanol - orig formalin
96629	Caryophylliidae	*Caryophyllia*	*diomedeae*	TAN0104/153	18/04/2001	42.7325	179.8985 W	1076	990	1	Ethanol - orig formalin
104981	Caryophylliidae	*Caryophyllia*	*profunda*	Z16074	02/02/1993	45.349	167.056	15	35	7	Ethanol - orig formalin
127291	Caryophylliidae	*Caryophyllia*	*diomedeae*	TAN0104/150	18/04/2001	42.7155556	179.9061111 W	1181	1004	4	Ethanol - orig formalin
127318	Caryophylliidae	*Caryophyllia*	*diomedeae*	TAN0104/116	17/04/2001	42.79816818	179.9818268	1000	922	5	Ethanol - orig formalin
127590	Caryophylliidae	*Caryophyllia*		TAN0104/333	20/04/2001	42.7183342	179.9095001 W	1075	1008	3	Ethanol - orig formalin
127383	Caryophylliidae	*Caryophyllia*	*diomedeae*	TAN0104/333	20/04/2001	42.7183342	179.9095001 W	1075	1008	3	Ethanol - orig formalin
127385	Caryophylliidae	*Caryophyllia*	*scobinosa*	Z10787	21/05/2001	34.88083267	179.0813293	1620		1	Ethanol - orig formalin
127390	Caryophylliidae	*Caryophyllia*	*diomedeae*	TAN0107/323	24/05/2001	36.1456667	178.2016667	924	712	2	Ethanol orig formalin
127392	Caryophylliidae	*Caryophyllia*	*profunda*	Z10804	23/05/2001	35.73783112	178.5075073	1045	500	2	Ethanol orig formalin
127393	Caryophylliidae	*Caryophyllia*	*diomedeae*	TAN0104/152	18/04/2001	42.729833	179.890333	1130	1000	1	Ethanol orig formalin
127394	Caryophylliidae	*Caryophyllia*	*diomedeae*	TAN0104/115	17/04/2001	42.80233383	179.9878387	1013	931	1	Ethanol orig formalin
127396	Caryophylliidae	*Caryophyllia*	*diomedeae*	TAN0107/225	23/05/2001	36.14733333	178.204	951	772	1	Ethanol orig formalin
127397	Caryophylliidae	*Caryophyllia*	*diomedeae*	TAN0104/149	18/04/2001	42.71699905	179.9600067	1162	980	6	Ethanol orig formalin
127400	Caryophylliidae	*Caryophyllia*	*diomedeae*	TAN0104/47	16/04/2001	42.79283524	179.9810028	950	900	7	Ethanol orig formalin

NIWA Invertebrate Collection Catalogue No.	Species name Family	Genus	species	Station code	Sample date	Position Latitude (S)	Longitude (all East unless shown as west=W)	Sample depth start (m)	finish (m)	Sample count	Preservation type
127401	Caryophylliidae	*Caryophyllia*	*profunda*	TAN0107/125	20/05/2001	35.74	178.5046667	641	243	1	Ethanol orig formalin
127402	Caryophylliidae	*Caryophyllia*	*diomedeae*	TAN0107/234	24/05/2001	36.1345	178.2011667	1140	698	2	Ethanol orig formalin
127404	Caryophylliidae	*Caryophyllia*	*profunda*	TAN0107/233	24/05/2001	36.13916779	178.1956635	520	367	1	Ethanol orig formalin
24781	Caryophylliidae	*Conotrochus*	*brunneus*	TAN0107/235	24/05/2001	36.1393333	178.196	672	367	1	Ethanol orig formalin
24782	Caryophylliidae	*Conotrochus*	*brunneus*	TAN0107/227	23/05/2001	36.13966667	178.1961667	603	365	12	Ethanol orig formalin
24785	Caryophylliidae	*Desmophyllum*	*dianthus*	TAN0104/152	18/04/2001	42.729833	179.890333	1130	1000	1	Ethanol orig formalin
118260	Caryophylliidae	*Desmophyllum*	*dianthus*	TAN1612/28	25/10/2016	29.285	177.857	499	615	1	Formalin
127327	Caryophylliidae	*Desmophyllum*	*dianthus*	TAN0107/227	23/05/2001	36.13966667	178.1961667	603	365	1	Ethanol orig formalin
47925	Caryophylliidae	*Desmophyllum*	*dianthus*	TRIP2699/17	02/10/2008	44.463333	174.89	1008	1087	1	Ethanol orig formalin
127403	Caryophylliidae	*Desmophyllum*	*dianthus*	TAN0107/234	24/05/2001	36.1345	178.2011667	1140	698	2	Ethanol orig formalin
104980	Caryophylliidae	*Desmophyllum*	*dianthus*	Z16074	02/02/1993	45.349	167.056	15	35	2	Ethanol orig formalin
88074	Caryophylliidae	*Desmophyllum*	*dianthus*	TAN0104/47	16/04/2001	42.79283524	179.9810028	950	900	4	Ethanol orig formalin
88075	Caryophylliidae	*Desmophyllum*	*dianthus*	TAN0104/153	18/04/2001	42.7325	179.8985	1076	990	8	Ethanol orig formalin
71137	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN0104/336	20/04/2001	42.7678333	179.9218333	955	890	1	Ethanol orig formalin
58350	Flabellidae	*Flabellum*		KAH0907/35	16/08/2009	34.45833333	173.1191667	110	108	1	Ethanol orig formalin
65125	Flabellidae	*Flabellum*	*knoxi*	TAN0601/71	10/01/2006	43.83283234	179.1889954	486	479	2	Ethanol orig formalin
88942	Flabellidae	*Flabellum*	*aotearoa*	Z9009	22/01/1998	37.22333145	176.239502	224		5	Ethanol orig formalin
88941	Flabellidae	*Flabellum*	*aotearoa*	KAH9907/50	05/06/1999	37.46900177	177.1161652	230	318	1	Ethanol orig formalin
147900	Caryophylliidae	*Goniocorella*	*dumosa*	TAN2001/81	22/01/2020	43.53183333	177.1036667	279	263	1	Formalin
81281	Caryophylliidae	*Goniocorella*	*dumosa*	TAN0104/116	17/04/2001	42.79816818	179.9818268	1000	922	1	Ethanol orig formalin
140313	Caryophylliidae	*Goniocorella*	*dumosa*	TAN1903/106	21/06/2019	43.3676667	179.4513333	396	396	1	Formalin
140326	Caryophylliidae	*Goniocorella*	*dumosa*	TAN1903/108	21/06/2019	43.3681667	179.4508333	387	380	1	Formalin
140346	Caryophylliidae	*Goniocorella*	*dumosa*	TAN1903/110	22/06/2019	43.3606667	179.7423333	461	450	1	Formalin

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140375	Caryophylliidae	*Goniocorella*	*dumosa*	TAN1903/153	25/06/2019	43.3653333	179.4505	390	390	1	Formalin
54068	Caryophylliidae	*Goniocorella*	*dumosa*	TAN0905/113	27/06/2009	44.1495	174.7568333	519	609	30	Ethanol orig formalin
3940	Oculinidae	*Javania*	*lamprotichum*	Z10706	17/04/2001	42.802333	179.987833	1013	931	1	Ethanol orig formalin
127387	Flabellidae	*Javania*	*lamprotichum*	TAN0104/333	20/04/2001	42.7183342	179.9095001	1075	1008	1	Ethanol orig formalin
127386	Caryophylliidae	*Labyrinthocyathus*	*langae*	TAN0104/333	20/04/2001	42.7183342	179.9095001	1075	1008	16	Ethanol orig formalin
118067	Oculinidae	*Madrepora*	*oculata*	TAN1612/4	23/10/2016	29.2836667	177.8576667	520		1	Formalin
127339	Oculinidae	*Madrepora*	*oculata*	TAN0104/336	20/04/2001	42.7678333	179.9218333	955	890	1	Ethanol orig formalin
3941	Oculinidae	*Madrepora*	*oculata*	Z10697	16/04/2001	42.79283	179.981	950	900	1	Ethanol orig formalin
3989	Oculinidae	*Madrepora*	*oculata*	Z10727	20/04/2001	42.76783	179.9218	955	890	1	Ethanol orig formalin
4027	Oculinidae	*Madrepora*	*oculata*	Z10698	16/04/2001	42.78617	179.9853	993	900	1	Formalin
3133	Flabellidae	*Polymyces*	*wellsi*	KAH0204/44	18/04/2002	34.26566696	174.1031647	850	840	1	Ethanol orig formalin
3935	Caryophylliidae	*Solenosmilia*	*variabilis*	TAN0104/333	20/04/2001	42.7183342	179.9095001	1075	1008	1	Ethanol orig formalin
89013	Caryophylliidae	*Solenosmilia*	*variabilis*	TAN0107/219	25/05/2001	35.73166667	178.5223333	1200	950	1	Ethanol orig formalin
89073	Caryophylliidae	*Solenosmilia*	*variabilis*	TAN0104/153	18/04/2001	42.7325	179.8985	1076	990	1	Ethanol orig formalin
89115	Caryophylliidae	*Solenosmilia*	*variabilis*	TAN0104/115	17/04/2001	42.80233383	179.9878387	1013	931	1	Ethanol orig formalin
89118	Caryophylliidae	*Solenosmilia*	*variabilis*	TAN0107/219	25/05/2001	35.73166667	178.5223333	1200	950	1	Ethanol orig formalin
89121	Caryophylliidae	*Solenosmilia*	*variabilis*	TAN0104/152	18/04/2001	42.729833	179.890333	1130	1000	1	Ethanol orig formalin
89124	Caryophylliidae	*Solenosmilia*	*variabilis*	TAN0104/333	20/04/2001	42.7183342	179.9095001	1075	1008	1	Ethanol orig formalin
39856	Caryophylliidae	*Solenosmilia*	*variabilis*	TAN0803/38	01/04/2008	50.09716667	163.4741667	1070	1123	1	Ethanol orig formalin
94164	Caryophylliidae	*Solenosmilia*	*variabilis*	TAN1402/31	11/02/2014	35.3168333	170.4515	1205	1600	1	Formalin
94409	Caryophylliidae	*Solenosmilia*	*variabilis*	TAN1402/97	21/02/2014	39.1958333	167.5898333	1082	1090	1	Formalin
86580	Caryophylliidae	*Solenosmilia*	*variabilis*	TAN0104/393	21/04/2001	42.79550171	179.9871674	1009	928	1	Ethanol orig formalin
78856	Caryophylliidae	*Stephanocyathus*	*platypus*	TAN1116/94	14/11/2011	42.83366667	178.6755	966	985	24	Ethanol orig formalin

NIWA Invertebrate Collection Catalogue No.	Species name Family	Genus	species	Station code	Sample date	Position Latitude (S)	Longitude (all East unless shown as west=W)	Sample depth start (m)	finish (m)	Sample count	Preservation type
66477	Caryophylliidae	*Stephanocyathus*	*platypus*	TRIP2955/82	15/10/2009	42.871667	175.398333	1046	1028	5	Ethanol orig formalin
144434	Caryophylliidae	*Stephanocyathus*	*platypus*	TAN1807/65	07/08/2018	42.012	169.507	971	974	1	Ethanol orig formalin
144435	Caryophylliidae	*Stephanocyathus*	*platypus*	TAN1807/62	07/08/2018	41.5276	169.5996	891	896	3	Ethanol orig formalin
144436	Caryophylliidae	*Stephanocyathus*	*platypus*	TAN1807/58	06/08/2018	42.0919	170.0015	915	920	1	Ethanol orig formalin
58351	Caryophylliidae	*Stephanocyathus*	*spiniger*	KAH0907/41	17/08/2009	34.59783333	173.37	174	204	1	Ethanol orig formalin
66486	Caryophylliidae	*Stephanocyathus*	*platypus*	TRIP2807/36	13/02/2009	42.775	175.625	1220	1224	1	Ethanol orig formalin
4568	Caryophylliidae	*Trochocyathus*	*cepulla*	K840	28/07/1974	30.29330063	178.4217072	398		1	Ethanol orig formalin
4569	Caryophylliidae	*Trochocyathus*	*cepulla*	P13	25/01/1977	32.17499924	167.353302	449		2	Ethanol orig formalin
127580	Caryophylliidae	*Vaughanella*	*multipalifera*	TAN0107/327	26/05/2001	41.55216667	175.7085	1355	1277	2	Ethanol orig formalin
94550	Caryophylliidae	*Solenosmilia*	*variabilis*	TAN1402/138	28/02/2014	41.5815	164.2551667	1223	1241	1	Ethanol orig formalin
94590	Caryophylliidae	*Solenosmilia*	*variabilis*	TAN1402/156	02/03/2014	41.3646667	164.419	1220	1250	1	Ethanol orig formalin
147900	Caryophylliidae	*Goniocorella*	*dumosa*	TAN2001/81	22/01/2020	43.53183333	177.1036667	279	263	1	Ethanol orig formalin
148101	Caryophylliidae	*Goniocorella*	*dumosa*	TAN2009/57	16/08/2020	44.159	174.554	486	659	10	Ethanol orig formalin
148157	Caryophylliidae	*Goniocorella*	*dumosa*	TAN2009/80	19/08/2020	44.136166	174.7211666	640	622	10	Ethanol orig formalin
148158	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN2009/80	19/08/2020	44.136166	174.7211666	640	622	10	Ethanol orig formalin
148159	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN2009/80	19/08/2020	44.136166	174.7211666	640	622	10	Ethanol orig formalin
154699	Caryophylliidae	*Caryophyllia*	*profunda*	TAN2009/80	19/08/2020	44.136166	174.7211666	640	622	3	Ethanol orig formalin

Appendix 2

Catalogue number, species name, sample position and depth data, sample count, and preservation method (ORIG = originally), data for Alcyonacea (gorgonian octocorals and true soft corals), selected from NIWA Invertebrate Collection Specify database *niwainvert* that have been or are currently preserved in formalin. From this list, gorgonian samples collected post 2010 and the endemic soft coral *T. tauhou* have been selected as recommended species for a reproduction study (see Table 4-3).

NIWA Invertebrate Collection Catalogue No.	Species name Family	Genus	species	Station code	Sample date	Position Latitude (S)	Longitude (all East unless shown as west=W)	Sample depth start (m)	finish (m)	Sample count	Preservation type
34476	Alcyonacea (to Order only)			TAN0705/82	09/04/2007	43.9737	179.633	526	533	1	Formalin
34791	Alcyonacea (to Order only)			TAN0705/98	10/04/2007	44.5602	178.477 W	1074	1081	5	Formalin
35305	Alcyonacea (to Order only)			TAN0604/15	29/05/2006	42.7575	179.992 W	830	1060	1	Formalin
36545	Alcyoniidae	*Anthomastus*		TAN0802/81	17/02/2008	76.594	176.828	369	365	1	Formalin
37970	Clavulariidae	*Clavularia*		TAN0802/182	29/02/2008	69.3867	178.712 W	415	410	10	Formalin
38582	Clavulariidae	*Clavularia*		TAN0802/245	07/03/2008	67.3833	179.844 W	760	559	1	Formalin
53721	Isididae			TAN0905/99	26/06/2009	44.1397	174.72 W	641	758	1	Formalin
64556	Alcyonacea (to Order only)			TAN1007/56	02/06/2010	35.3603	178.5088	1270	1267	1	Formalin
64711	Alcyonacea (to Order only)			TAN1007/100	06/06/2010	35.4347	178.6302	1402	1530	1	Formalin
64768	Isididae	*Lepidisis*		TAN1007/104	06/06/2010	35.3622	178.5258	1287	1378	1	Formalin
64862	Isididae			TAN1007/109	07/06/2010	35.3503	178.5462	1171	1240	1	Formalin
72542	Primnoidae			TAN1104/59	11/03/2011	35.3595	178.5105	1270	1410	1	Formalin
83450	Isididae	*Lepidisis*		TAN1206/176	01/05/2012	37.2597	178.016	1540	1497	1	Formalin
86283	Alcyoniidae	*Anthomastus*		TAN1213/27	19/10/2012	30.6953	179.37 W	1261	1644	1	Formalin

NIWA Invertebrate Collection Catalogue No.	Species name Family	Genus	species	Station code	Sample date	Position Latitude (S)	Longitude (all East unless shown as west=W)	Sample depth start (m)	finish (m)	Sample count	Preservation type
148162	Primnoidae	*Thouarella*		TAN2009/80	19/08/2020	44.1362	174.721 W	640	622	1	Formalin
148163	Alcyonacea (to Order only)			TAN2009/80	19/08/2020	44.1362	174.721 W	640	622	1	Formalin
210	Isididae	*Keratoisis*	*glaesa*	C632	27/05/1961	39.2333	172.0167	406		1	Ethanol - orig formalin
212	Isididae	*Keratoisis*	*tangentis*	C632	27/05/1961	39.2333	172.0167	406		4	Ethanol - orig formalin
3315	Paragorgiidae	*Paragorgia*	*alisonae*	Z9595	27/11/1998	48.0167	166.1	940	1180	1	Ethanol - orig formalin
3316	Paragorgiidae	*Paragorgia*	*alisonae*	Z8981	05/12/1997	44.9613	174.1872	1041	1052	1	Ethanol - orig formalin
3317	Paragorgiidae	*Paragorgia*	*alisonae*	Z9583	25/11/1998	48.0335	166.1002	935		1	Ethanol - orig formalin
3977	Alcyoniidae			Z9688	27/01/1999	34.3745	172.7013	53		2	Ethanol - orig formalin
3987	Alcyoniidae	*Anthomastus*		Z10697	16/04/2001	42.7928	179.981 W	950	900	17	Ethanol - orig formalin
3988	Alcyoniidae	*Anthomastus*		TAN0104/337	20/04/2001	42.7667	179.923 W	970	900	2	Ethanol - orig formalin
28353	Alcyoniidae	*Anthomastus*		TAN0602/394	06/03/2006	67.3502	179.878 W	540	600	3	Ethanol - orig formalin
30536	Taiaroiidae	*Taiaroa*	*tauhou*	TAN0705/15	03/04/2007	45.0583	175.4743	1238	1258	1	Ethanol - orig formalin
32014	Primnoidae	*Thouarella*		SO191-2/165	22/02/2007	40.0532	177.8182	1109	1112	1	Ethanol - orig formalin
34524	Taiaroiidae	*Taiaroa*	*tauhou*	TAN0705/254	24/04/2007	43.5413	178.505	348	350	11	Ethanol - orig formalin
34526	Taiaroiidae	*Taiaroa*	*tauhou*	TAN0705/257	24/04/2007	43.2642	178.5142	402	407	1	Ethanol - orig formalin
34793	Alcyoniidae	*Heteropolypus*		TAN0705/10	03/04/2007	44.1287	174.8445	513	517	2	Ethanol - orig formalin
34863	Alcyoniidae	*Anthomastus*		TAN0705/213	21/04/2007	42.682	177.212 W	1284	1298	1	Ethanol - orig formalin
34867	Taiaroiidae	*Taiaroa*	*tauhou*	TAN0705/15	03/04/2007	45.0583	175.4743	1238	1258	7	Ethanol - orig formalin
34962	Alcyoniidae	*Paraminabea*		TAN0413/130	14/11/2004	37.3557	177.0997	260	280	1	Ethanol - orig formalin
35284	Primnoidae	*Thouarella*		TAN0604/108	06/06/2006	43.5328	179.628	375	381	3	Ethanol - orig formalin
35285	Alcyoniidae	*Anthomastus*		TAN0604/106	05/06/2006	42.7268	179.9 W	1030	1156	1	Ethanol - orig formalin
35327	Alcyonacea (to Order only)			TAN0604/30	30/05/2006	42.765	179.988 W	951	1076	1	Ethanol - orig formalin

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35338	Alcyonacea (to Order only)			TAN0604/21	29/05/2006	42.766	179.926 W	906	1061	1	Ethanol - orig formalin
35341	Taiaroiidae	*Taiaroa*	*tauhou*	TAN0705/27	04/04/2007	43.8353	174.6793	507	510	1	Ethanol - orig formalin
35344	Taiaroiidae	*Taiaroa*	*tauhou*	TAN0705/287	27/04/2007	43.7257	174.458	552	552	6	Ethanol - orig formalin
36539	Alcyonacea (to Order only)			TAN0802/81	17/02/2008	76.594	176.828	369	365	1	Ethanol - orig formalin
38567	Alcyonacea (to Order only)			TAN0802/245	07/03/2008	67.3833	179.844 W	760	559	10	Ethanol - orig formalin
39721	Alcyonacea (to Order only)			TAN0803/33	01/04/2008	50.0905	163.4822	1077	1408	1	Ethanol - orig formalin
41982	Primnoidae	*Thouarella*		TAN0402/144	26/02/2004	72.0343	170.9143	273	273	1	Ethanol - orig formalin
41984	Alcyoniidae	*Alcyonium*		TAN0402/39	10/02/2004	71.755	171.1425	251	253	1	Ethanol - orig formalin
41986	Primnoidae	*Arntzia*	*gracilis*	TAN0402/144	26/02/2004	72.0343	170.9143	273	273	1	Ethanol - orig formalin
41987	Alcyonacea (to Order only)			TAN0616/9	04/11/2006	40.0395	178.1435	748		1	Ethanol - orig formalin
41988	Primnoidae	*Primnoella*		TAN0402/205	29/02/2004	71.1632	171.0477	1014	1014	1	Ethanol - orig formalin
41991	Primnoidae	*Thouarella*		TAN0402/52	12/02/2004	72.3368	170.3942	154	153	1	Ethanol - orig formalin
41992	Alcyonacea (to Order only)			TAN0402/156	26/02/2004	71.9927	172.207	675	675	1	Ethanol - orig formalin
41993	Alcyoniidae	*Alcyonium*		TAN0402/127	19/02/2004	71.3237	170.409	85	85	1	Ethanol - orig formalin
43347	Taiaroiidae	*Taiaroa*	*tauhou*	TAN0705/90	10/04/2007	44.1073	178.55 W	459	460	1	Ethanol - orig formalin
46371	Alcyonacea (to Order only)			TRIP2614/37	12/04/2008	49.8033	175.8683	1049	1160	1	Ethanol - orig formalin
46372	Primnoidae	*Parastenella*	*spinosa*	TRIP2494/14	02/09/2007	47.5467	177.8283	867	915	1	Ethanol - orig formalin
46373	Isididae	*Acanella*		TRIP2614/218	09/05/2008	48.4283	175.0117	890	970	1	Ethanol - orig formalin

NIWA Invertebrate Collection Catalogue No.	Species name Family	Genus	species	Station code	Sample date	Position Latitude (S)	Longitude (all East unless shown as west=W)	Sample depth start (m)	finish (m)	Sample count	Preservation type
46374	Isididae	*Acanella*		TRIP2571/161	20/03/2008	49.975	163.7683	829	992	1	Ethanol - orig formalin
46375	Isididae	*Keratoisis*		TRIP2626/66	26/05/2008	44.725	176.805 W	716	1072	1	Ethanol - orig formalin
46376	Isididae	*Lepidisis*		TRIP2608/117	07/05/2008	43.9133	174.672 W	668		1	Ethanol - orig formalin
46377	Paragorgiidae	*Paragorgia*	*arborea*	TRIP2571/53	29/02/2008	50	176.06	952	1118	1	Ethanol - orig formalin
48469	Alcyoniidae	*Heteropolypus*		TAN0705/24	04/04/2007	44.1208	174.8432	512	513	1	Ethanol - orig formalin
49745	Primnoidae	*Arntzia*	*gracilis*	E177	14/01/1965	75.9833	168.1833	190		1	Ethanol - orig formalin
61920	Primnoidae	*Primnoa*	*notialis*	TRIP3065/214	09/03/2010	45.0317	175.495	1070	1100	1	Ethanol - orig formalin
61980	Primnoidae	*Primnoa*	*notialis*	TRIP3077/127	31/03/2010	48.8167	175.3833	769	767	1	Ethanol - orig formalin
63004	Primnoidae	*Thouarella*		TAN1004/2	15/04/2010	41.6712	175.625	640	635	1	Ethanol - orig formalin
64511	Chrysogorgiidae	*Iridogorgia*		TAN1007/54	02/06/2010	35.3542	178.5262	1166	1209	1	Ethanol - orig formalin
65588	Clavulariidae	*Clavularia*		TRIP2862/160	15/06/2009	42.7633	176.943 W	1069	1074	1	Ethanol - orig formalin
66274	Paragorgiidae	*Paragorgia*	*arborea*	TRIP3028/136	10/01/2010	44.4533	178.602 W	735		1	Ethanol - orig formalin
85634	Taiaroiidae	*Taiaroa*	*tauhou*	TAN1208/70	26/06/2012	43.7527	179.077 W	397	397	1	Ethanol - orig formalin
85885	Taiaroiidae	*Taiaroa*	*tauhou*	TAN1208/35	19/06/2012	42.9108	179.9618	758	751	5	Ethanol - orig formalin
85907	Taiaroiidae	*Taiaroa*	*tauhou*	TAN1208/34	18/06/2012	42.8675	179.745 W	789	778	1	Ethanol - orig formalin
86060	Alcyoniidae			TAN1213/18	18/10/2012	30.1865	179.7218	380	440	4	Ethanol - orig formalin
86071	Nephtheidae			TAN1213/18	18/10/2012	30.1865	179.7218	380	440	16	Ethanol - orig formalin
86072	Nephtheidae			TAN1213/18	18/10/2012	30.1865	179.7218	380	440	28	Ethanol - orig formalin
86074	Nephtheidae			TAN1213/18	18/10/2012	30.1865	179.7218	380	440	1	Ethanol - orig formalin
86075	Nephtheidae			TAN1213/18	18/10/2012	30.1865	179.7218	380	440	1	Ethanol - orig formalin
86137	Nephtheidae			TAN1213/19	18/10/2012	30.1773	179.7369	387	422	13	Ethanol - orig formalin
86138	Nephtheidae			TAN1213/19	18/10/2012	30.1773	179.7369	387	422	2	Ethanol - orig formalin
90860	Taiaroiidae	*Taiaroa*								1	Ethanol - orig formalin

NIWA Invertebrate Collection Catalogue No.	Species name Family	Genus	species	Station code	Sample date	Position Latitude (S)	Longitude (all East unless shown as west=W)	Sample depth start (m)	finish (m)	Sample count	Preservation type
90861	Taiaroiidae	*Taiaroa*								1	Ethanol - orig formalin
91089	Anthothelidae	*Solenocaulon*		Z10660	29/12/2000	43.5477	174.0463	373		1	Ethanol - orig formalin
97833	Primnoidae			TAN0402/234	04/03/2004	67.4463	163.8098	477	526	1	Ethanol - orig formalin
103123	Alcyonacea (to Order only)			TAN0402/152	26/02/2004	71.9947	172.1342	515	494	10	Ethanol - orig formalin
103124	Alcyonacea (to Order only)			TAN0402/88	14/02/2004	72.0977	172.9302	515	515	1	Ethanol - orig formalin
103134	Alcyonacea (to Order only)			TAN0402/157	26/02/2004	71.9853	172.1785	737	718	25	Ethanol - orig formalin
103135	Alcyonacea (to Order only)			TAN0402/157	26/02/2004	71.9853	172.1785	737	718	25	Ethanol - orig formalin
103136	Alcyonacea (to Order only)			TAN0402/154	26/02/2004	72.0013	172.2223	536	586	10	Ethanol - orig formalin
103137	Alcyonacea (to Order only)			TAN0402/153	26/02/2004	72.0085	172.2227	540	540	5	Ethanol - orig formalin
103138	Alcyonacea (to Order only)			TAN0402/202	29/02/2004	71.1553	171.0923	930	940	5	Ethanol - orig formalin
103247	Isididae			TAN0402/49	12/02/2004	72.33	170.3933	158	158	1	Ethanol - orig formalin
104848	Primnoidae	*Metafannyella*	*moseleyi*	TAN0308/97	28/05/2003	33.771	167.325	273	260	1	Ethanol - orig formalin
114464	Alcyonacea (to Order only)			TAN0307/59	29/04/2003	49.3105	179.798	1506	1476		Ethanol - orig formalin
114465	Alcyonacea (to Order only)			TAN0307/64	29/04/2003	48.6735	179.6225	757	764		Ethanol - orig formalin
125218	Primnoidae	*Thouarella*		Z10652	01/03/2001	66.6328	163.0418	183		5	Ethanol - orig formalin
131909	Alcyonacea (to Order only)			TRIP5613/96	01/05/2019	42.9233	175.2317	560	522	1	Ethanol - orig formalin
144437	Isididae			TAN1807/58	06/08/2018	42.0919	170.0015	915	920	1	Ethanol - orig formalin

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148160	Isididae	*Minuisis*		TAN2009/80	19/08/2020	44.1362	174.721 W	640	622	2	Ethanol - orig formalin
148162	Primnoidae	*Thouarella*		TAN2009/80	19/08/2020	44.1362	174.721 W	640	622	1	Ethanol - orig formalin
148164	Telestidae	*Telesto*		TAN2009/80	19/08/2020	44.1362	174.721 W	640	622	5	Ethanol - orig formalin
154698	Primnoidae	*Thouarella*		TAN2009/80	19/08/2020	44.1362	174.721 W	640	622	1	Ethanol - orig formalin